VOLUME 42 JANUARY, 1952 NUMBER 1

Phytopathology

UNIVERSITY OF MICHIGAN
FEB 13 1952
SCIENCE LIBRARY

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

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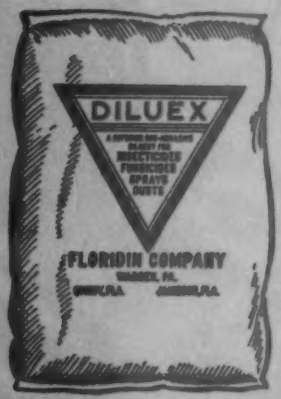

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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTY-THIRD ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, CINCINNATI, OHIO, DECEMBER 10, 11, and 12, 1951

Sclerotium rolfsii, a destructive pathogen of alfalfa and Ladino clover. ALLISON, J. LEWIS. Alfalfa and Ladino clover, perennial forage legumes recently introduced into southeastern United States, have been found severely damaged by *Sclerotium rolfsii*. *S. rolfsii* has long been known as a soil-inhabiting fungus causing a disease, commonly called southern blight, on many field and horticultural crops. Typical blight symptoms occur on both legumes, and established stands frequently are severely depleted by the disease. Hot, humid weather favors its spread and development. Single sclerotial isolates from alfalfa and Ladino clover were compared on potato-dextrose agar with isolates from annual lespedeza and soybean. All isolates were morphologically similar and produced abundant sclerotia in culture. Optimum temperature for growth of all isolates was 25° to 30° C. Pathogenicity of each isolate was determined on the legume from which it was obtained and by cross inoculation to the other legumes. All isolates were pathogenic on each of the legumes. Optimum temperature for disease development was 25° to 30° C. On the basis of the results obtained the legumes tested were rated in respect to their susceptibility to *S. rolfsii:* 1) Very susceptible — Ladino clover; 2) susceptible — alfalfa, annual lespedeza, and soybean.

Relation of solanine content of tomato fruits to colorization by Colletotrichum phomoides. ALLISON, PATRICIA VAN BURGH. Growth in length of hyphae of *Colletotrichum phomoides*, isolate 76-2, was much less in expressed sap from small, green Rutgers tomato fruits than in sap from ripe fruits. Crude solanine was extracted from small, green fruits by the method of Bömer and Mattis. None was obtained by the same method from mature fruits. The concentration of the material in the outer 1 to 2 mm. of tissue was greater than in the remainder of the fruit. Conidia were suspended in expressed sap from green fruits, sap from ripe fruits, and in ripe fruit sap containing 500, 1000, and 1500 p.p.m. of solanine obtained from green tomato fruits. All levels of solanine and the green fruit sap had inhibitory effects on the fungus as compared with ripe fruit sap alone. Solanine obtained from green tomato fruits was compared with a material obtained from potato sprouts by the same extraction method. Both were tested at 500, 1000, and 1500 p.p.m. in 2 per cent glucose. The tomato preparation was much more toxic than the potato preparation. Tomato solanine in green fruits may account, at least in part, for the failure of the anthracnose organism to colonize green fruits after invasion has occurred.

Control of bean anthracnose in navy pea beans by seed treatment. ANDERSEN, AXEL L. Of 30 fungicides tested, Vancide 51 (sodium salts of dimethyl dithiocarbamic acid and of 2-mercaptobenzothiazole) was the only one that effectively controlled bean anthracnose (*Colletotrichum lindemuthianum*) when used on visibly infected navy pea bean seeds in greenhouse tests. Satisfactory control was obtained by soaking the infected seed in a 7.5 or 15 per cent solution of the active ingredients of Vancide 51 for 40 min. This treatment resulted in wrinkling and sloughing of the seed coats, imbibition of large quantities of liquid, and about a 50 per cent reduction in stand. Concentrations of 7.5 or 15 per cent Vancide 51 (100 per cent active ingredients) in a 2 to 3 per cent Methocel slurry were more satisfactory. With the concentrations mentioned, complete control was obtained in the greenhouse when 20 ml. of slurry were applied to the seed for 10 to 40 min. There were little wrinkling and sloughing of the seed coat, little imbibition of liquid by the seed, and no impairment of germination. Furthermore, only 10 min. were required to dry the seed. In field experiments, 5 to 10 per cent concentrations of the fungicide in Methocel were used with a 20-min. treatment. Nearly complete control was obtained in all the treated plots.

Physiologic races of the Fusaria causing wilts of the Cruciferae. ARMSTRONG, G. M., AND JOANNE K. ARMSTRONG. Plants of radish, stock, and various members of the cabbage tribe were inoculated with single-spore isolates of wilt Fusaria from cabbage and radish. The cabbage *Fusarium* was highly pathogenic to radish and to cabbage and some other members of the cabbage tribe, and moderately so to one variety of stock. The radish *Fusarium* was highly pathogenic to radish and one variety of stock, moderately so to a variety of brussels sprouts and one of kale, and less so or nonpathogenic to other members of the cabbage tribe. The cabbage and radish Fusaria produced no wilting of wilt-susceptible plants of 18 genera not in the Cruciferae. Inoculation of one variety each of cabbage and radish with 18 different wilt Fusaria from other hosts failed to produce any symptoms of wilt. It is proposed that the wilt Fusaria from cabbage and from radish, and probably the one from stock, be designated as physiologic races of *F. conglutinans* and not forms of *F. oxysporum* since there is a common host. The *Fusarium* originally described from cabbage would be race 1; the one with a high degree of pathogenicity chiefly for radish, race 2; and the one from stock probably race 3.

Rhizoctonia aerial blight of soybeans in Louisiana. ATKINS, J. G., JR., AND W. D. LEWIS. Rhizoctonia aerial blight was observed in soybean variety plots at Baton Rouge in 1950 and 1951. This disease was more severe in 1951 on certain varieties than the other common foliage diseases. Varieties differed considerably in susceptibility, Dortchsoy 31 and Ogden having been conspicuously more susceptible than others in the field. Symptoms ranged from definite spots to complete killing of leaves, petioles, pods, and young stems. Severely affected plants were nearly defoliated. Leaves 2 or 3 ft. above the soil surface were frequently attacked. Typical Rhizoctonia mycelium developed on or between infected leaflets. Small sclerotia of the *R. microsclerotia* type were produced abundantly in 1951. In controlled tests, isolates caused leaf blight on soybeans, snap beans, lima beans, cowpeas, fescue, trefoil, Lespedeza, fig, and tung. In addition to the microsclerotia type of Rhizoctonia, other types can cause leaf blight on soybeans. The disease, pathogen, and conditions favoring development are the same as for web blight or similar blights of snap beans and certain other Southern crops as reported by various workers. In areas where favorable environmental conditions may prevail, this blight should be considered a potentially destructive disease.

Some effects of the citrus-root nematode on the growth of orange and lemon trees. BAINES, R. C., AND O. F. CLARKE. The growth of young orange and lemon trees in eight pot-culture experiments was reduced 10 to 50 per cent following inoculation with the citrus-root nematode. The percentage decrease of growth of the trees was not always proportional to the degree of infestation, which suggests that undetermined factors may affect the tolerance of trees to this nematode. In one experiment the young leaves on eight of nine inoculated Valencia orange trees on sweet-orange rootstock developed a fine mottle. The top two feet of California citrus soils frequently contain 2 to 7 million citrus-root nematode larvae per cu. ft. Citrus trees planted on such soils become severely infested and grow poorly.

A new method for quick determination of the oak wilt fungus. BARNETT, H. L. *Chalara quercina* can be positively identified in less than one week using a technique based upon the characteristic ability of the fungus to sporulate quickly in submerged liquid culture. An easier and quicker

method has been found, based on a special agar medium, the characteristic appearance of the mycelium, and the quick sporulating habit of the fungus. The medium is composed of: glucose, 3 gm.; phenylalanine, 0.5 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 0.5 gm.; sulfates of Fe, Mn, Zn, each 0.2 mgm.; biotin, 5μgm.; agar, 20 gm.; distilled water, 1 liter. The pH should be near 6.0. The temperature of incubation should not be over 25° C. On this medium the mycelium of *C. quercina* grows out from oak chips after 3 to 5 days. It is sparse and may be examined directly with the miscroscope. Typical conidiophores and conidia are usually produced quickly near the chips, either on or under the surface of the agar. Characteristic branches which probably represent abortive conidiophores are valuable clues in identification. Mycelium similar to that of *Chalara* but which fails to produce spores on agar may be transferred to liquid medium for certain identification.

Physiological factors affecting growth and sporulation of Chalara quercina in culture. BARNETT, H. L., AND V. G. LILLY. *Chalara quercina* was highly deficient for biotin when grown in liquid glucose-casein hydrolysate medium. The fungus grew and sporulated well within the range 15°–25° C.; sporulation ceased near 28° C. No growth occurred below pH 3.0; best growth was made between 6.0 and 7.0. Sugars utilized well (in order based on fungus growth in liquid) were: mannose, sucrose, glucose, cellobiose, maltose, galactose, xylose, and fructose. Lactose, raffinose, and arabinose were utilized poorly. Only a trace of growth was made in sorbose medium. Nitrate nitrogen was not utilized. Growth was slow on ammonium nitrogen, although early sporulation was heavy in liquid media. Various sources of organic nitrogen were utilized well. Growth and sporulation were compared on agar and liquid media containing single amino acids. Growth was most rapid on asparagine, aspartic acid, and glutamic acid; intermediate on proline, alanine, valine, and phenylalanine; and slight on glycine, histidine, lysine, and serine. Sporulation was greater on phenylalanine than on any other agar medium. Quick sporulation was favored by a medium low in nitrogen and sugar. Cultures usually produced spores more quickly and abundantly in liquid media than on the same media solidified with agar.

Physiologic specialization in Puccinia menthae. BAXTER, JOHN W. The mint rust organism, *Puccinia menthae* Pers., is composed of at least ten physiologic races, differentiated on the basis of their ability to infect species in eight genera of the Labiatae. The race infecting *Mentha spicata, M. arvensis,* and *M. rotundifolia* has been designated race 1. Race 2 infects *Mentha piperita, M. arvensis,* and *M. longifolia.* The other eight races are specialized on *Monarda didyma, Monarda punctata, Monarda fistulosa, Blephilia hirsuta, Cunila origanoides, Satureja vulgaris, Pycnanthemum* spp., and *Monardella* spp.

Plugging of vessels associated with oak wilt development. BECKMAN, C. H., J. E. KUNTZ, A. J. RIKER, AND J. G. BERBEE. Certain host responses associated with the development of oak wilt were studied. The rates of movement of liquid in xylem vessels of healthy and diseased northern pin oaks were determined with solutions of radioactive rubidium ($Rb_2^{86}CO_3$). An excess of potassium chloride greatly reduced rubidium absorption. Rubidium moved upward approximately 1 foot per min. in healthy trees as well as in inoculated trees prior to symptom development. However, movement was reduced abruptly by 90 per cent and 99 per cent at the time of incipient and severe wilt, respectively. A similar reduction occurred in water flow through uniform 4-in. twig sections. Likewise, 3 weeks after inoculation this plugging occurred in trees from which all leaves were cut off 0, 1, 2, or 3 weeks following inoculation. No plugging occurred in uninoculated but defoliated controls. In general, either no bud growth appeared or buds shriveled 3 weeks after defoliation in inoculated trees. Many tyloses occurred in wilting twigs. As yet, no important plugging of roots has been observed. These results indicate 1) that vessel plugging is a direct response of the host to infection, 2) that such obstruction may limit the available water supply, and 3) that this shortage may contribute to leaf symptoms.

Reducing mold counts in canned or frozen black raspberries. BENEKE, E. S., AND WILLIAM J. YOUNG. Attempts were made in 1951 to control the high mold counts occurring in canned or frozen black raspberries harvested under hot, humid weather conditions. Sprays were applied 14 and 8 days before harvest in addition to the regular spray schedule. Actidione, 341 SC, nabam, ferbam, and Phygon XL were used alone and in combination with a spreader, Triton B 1956. The Howard mold count method was used to determine the effectiveness of the sprays. Ferbam and actidione consistently showed a lower mold count than the other materials. However, the reduction under hot, humid conditions was not enough to justify the use of these special sprays for black raspberries.

Recovery of water pimpernel, Samolus parviflorus Raf., from curly top and the reaction of recovered plants to inoculation with different virus strains. BENNETT, C. W. Water pimpernel shows a wide range in severity of curly-top symptoms varying from veinclearing, mild leaf rolling, and slight stunting induced by the less virulent strains, to severe leaf curling, dwarfing, and even death of affected plants induced by the more severe strains. Plants that survive eventually recover to a marked or high degree regardless of the strain of virus involved. However, plants that recover from the more severe strains are lower in vigor than healthy plants. Recovery appears to be associated with an appreciable decrease in virus concentration in the recovered plant. Resistance of recovered plants to infection and injury by other strains was tested in all possible combinations of 10 virus strains. Plants that recovered from each of the 10 strains were inoculated also with each of seven other strains with a moderate to high degree of virulence. In these tests, plants that recovered from mild strains were not protected to any measurable degree against either infection or injury by severe strains. In general, recovered plants were protected against injury by all strains of the virus that were less virulent than the strain from which the plant had recovered.

Effect of carborundum on infectivity of tobacco mosaic virus. BERAHA, LOUIS, AND H. H. THORNBERRY. The effect of carborundum on the infectivity of tobacco mosaic virus in nonpurified juice diluted 10^{-3} is influenced by the hydrogen-ion concentration and the buffer salt concentration of the virus solutions used as inoculum. The optimal concentration of phosphate salt for infection on Scotia beans (*Phaseolus vulgaris* L.) was 0.1 molar with or without carborundum. The optimal hydrogen-ion concentration for infection with the aid of carborundum was pH 7.2 without phosphate buffer and pH 8.5 with 0.1 molar phosphate buffer. Virus adsorption by the carborundum particles is related to the pH of the suspension (more adsorption with greater acidity). Nonadsorbed virus is responsible for infection under optimal conditions of pH 8.5 and 0.1 molar phosphated buffer. Thus, free virus causes the infection and the carborundum particles serve as an aid to inoculation, presumedly by providing suitable wounds. Increased infections by addition of aluminum oxide or silicon dioxide were relative to the mesh size; i.e., within the range studied, the greater the mesh size designation the greater the increase in infectivity. There was essentially no difference between aluminum oxide and silicon dioxide at any given mesh size. Dilution end-point of the juice was increased from 10^{-5} in water to 10^{-7} in 0.1 M. PO_4 with carborundum.

Turnip mosaic in Ontario. BERKELEY, G. H., AND M. WEINTRAUB. A mosaic of rutabaga was first observed near Walkerton, Ontario, in 1946. The virus nature of the disease has been established. The virus was readily transmitted by juice-transfer–carborundum methods to *Nicotiana tabacum, N. glutinosa,* turnip, cabbage, cauliflower, rape, Chinese cabbage, mustard, stock, wallflower, petunia, peppergrass, and shepherd's purse. Radish was insusceptible. On tobacco only primary symptoms developed, whereas the other hosts became systemically affected. The virus has been purified by chemical means and its isoelectric point

was approximately pH 4.5. Amoeboid inclusion bodies up to 30μ × 10μ were readily found in subveinal strippings of turnip epidermis stained with Trypan blue and phloxine. The thermal death point of the virus was 62°C. (10 min.) and its longevity *in vitro* was between 24 and 36 hr. A comparison with other virus diseases of crucifers would indicate that the Ontario disease is similar to the turnip mosaic reported by Chamberlain in New Zealand and most likely belongs to the turnip virus 1 group.

On the viral nature of papaya bunchy top. BIRD, JULIO, AND JOSÉ ADSUAR. Previous studies gave indications of the viral nature of papaya bunchy top, but the evidence was inconclusive. Failure of latex flow, as determined by pricking, shortly follows penetration of tissues by the causal agent. Downward spread of the virus is very slow and affected plants can be cured by cutting back the stem immediately below the affected portion. Evidence obtained through the use of the leafhopper, *Empoasca papayae* Oman, associated with the transmission of the virus, was limited by inability to secure nonviruliferous insect stock cultures. Evidence that the causal agent is capable of reproducing within the host plant was demonstrated by transmission of the causal agent in series through one plant after another by grafting. A single leafhopper was capable of transmitting the viral agent. Healthy plants upon which leafhoppers fed for a single day developed symptoms one and one-half months later.

Partial purification and electron microscopy of tomato spotted-wilt virus. BLACK, L. M., M. K. BRAKKE, AND A. E. VATTER. *Lethum australiense* Holmes was obtained from three phytopathologists. Each collection produced typical symptoms of tomato spotted wilt in *Lycopersicon esculentum*, *Callistephus chinensis*, *Tropaeolum majus*, *Nicotiana tabacum*, and *N. glutinosa*. A collection which produced good systemic symptoms in *N. rustica* and the greatest number of necrotic lesions on Turkish tobacco was selected for study. The longevity *in vitro* and thermal inactivation point were characteristic of tomato spotted-wilt virus. Plants of *N. rustica* grown under low light intensity were used as a virus source at the time of maximum infectivity. Laboratory work was done at 0° C. When Turkish tobacco was used as test plants, infectivity studies of the sedimentation of the virus in capillary tubes revealed that it aggregated in extracts in a solution of 0.1 M neutral phosphate buffer and 0.01 M Na_2SO_3. The virus was dispersed by a solution of low ionic strength containing 0.01 per cent Tween-80. Cycles of low-speed and high-speed centrifugation with aggregated and dispersed virus were followed by density-gradient centrifugation at 10,000 r.p.m., resulting in visible zones possessing about half the infectivity. Dried virus from the zones was seen under the electron microscope as oblate ellipsoids varying from about 90 to about 120 millimicrons in the greater diameter.

Effect of nutritive sprays on the development of Fusarium wilt of tomato. BLOOM, JAMES R. Bonny Best tomato plants growing in Hoagland's balanced solution in quartz sand continuous drip culture were inoculated with *Fusarium oxysporum* f. *lycopersici* (Sacc.) S. & H. by removing plants from the sand and dipping their roots in a suspension of mycelium and spores. After recovery from transplanting, the plants were given supplementary nutrient by spraying the foliage with various concentrations of sucrose, urea plus sucrose, KCl plus sucrose, and NaH_2PO_4 plus sucrose. With sucrose alone and with sucrose plus urea the disease index increased with rise in concentration from 0.1 to 0.7 molar. A decided but less gradual rise occurred with KCl plus sucrose. NaH_2PO_4 plus sucrose had no significant effect.

Control of black rot and downy mildew of grapes with concentrate sprays, dry dusts, and wet dusts. BRAUN, ALVIN J. Concentrate sprays and dry and wet dust applications have been compared with conventional hydraulic spray applications in tests in New York State vineyards since 1946. Modifications in the design of the applicator, based upon experience gained during the earlier tests, resulted in the development of a machine considered to be adequately adapted for vineyard use. With the present machine a 4× concentration is delivered under 300 p.s.i. pressure through a single No. 4 spray disk into the air blast from a 4-inch circular outlet. The concentrate spray or dust is directed backward and upward at an angle toward the underside of the canopy formed by the vine. The capacity requirements of a concentrate machine of the type used in these tests are such that construction costs could be kept low enough to compete with the regular type sprayers. Disease control data from nine separate tests indicate that the control with concentrate sprays (25 gal. per acre) is comparable with that obtained by an equal number of regular spray applications (100 gal. per acre). Dry and wet dust applications were equally effective in tests where disease development in the check plots was moderate.

New hosts for the oak wilt fungus, Chalara quercina Henry. BRETZ, T. W. *Lithocarpus densiflorus* (tanoak), *Castanopsis sempervirens* (bush chinquapin), *Castanea sativa* (European chestnut), and *Quercus acutissima* and *Quercus dentata* (Asiatic oak species) have been found to be susceptible to the oak wilt fungus, *Chalara quercina* Henry, in greenhouse inoculation tests. Potted trees, 1 to 3 years old, were used. Two isolates, one obtained from naturally infected northern red oak, the other from diseased Chinese chestnut, provided the inoculum. No differences in the pathogenicity of the isolates were observed. The progression of symptom development was similar in all species. A pronounced upward and inward rolling of the leaf blades on the leader and at the terminals of lateral branches, accompanied by a marked stiffening of the foliage, was the first evidence of infection. This was followed by a progressive loss of leaf color and ultimate death and browning of leaf tissues. Symptom development progressed downward until the entire plant became involved. The foliage in all instances remained attached after death. In some cases a pronounced suckering developed from the lower portions of the main stem prior to death of the plants. Discoloration of the wood near the cambium region usually accompanied the development of the foliage symptoms. The causal fungus was reisolated from each species inoculated.

The perithecial stage of Chalara quercina Henry. BRETZ, T. W. When compatible strains of *Chalara quercina* Henry are grown together on certain culture media, fertile perithecia of a species of *Ceratostomella* begin to form in 7 to 10 days. They are black, flask-shaped, and inconspicuous, with the spherical base 240–380 μ in diameter almost wholly embedded in the subiculum. The erect beaks, 250–450 μ long, which protrude above the subiculum are black and are terminated by a cluster or fringe of colorless filaments. The asci are globose to subglobose, 7–10 μ in the greatest diameter, and are evanescent. The ascospores are hyaline, one-celled, elliptical, and slightly curved, 2–3 μ wide by 5–10 μ long, collecting at the ostiole in a sticky, creamy-white mass that does not disintegrate in water. The ascospores germinate rapidly in 2 per cent dextrose solution at 25° C., the single germ tube produced giving rise to characteristic *C. quercina* endoconidia. Typical oak wilt symptoms have been obtained in susceptible species inoculated directly with ascospores and with single ascospore isolates. Asexual, thick-walled, olivaceous spores of variable shape and size, formed endogenously or intercalarily in hyphae which may also produce the hyaline endoconidia, have been found in perithecium-forming cultures. Neither perithecia nor the colored, thick-walled spores have been reported in nature.

Sugar and amino acid composition and variations found in urediospores of races of cereal rusts. BROYLES, JAMES W. Urediospores of races 15B, 36, 40, and 56 in *Puccinia graminis tritici*, races 7 and 8 of *P. graminis avenae*, and race 57 of *P. coronata* were analyzed by paper partition chromatography. Only traces of reducing sugars were detected in the ethanol-soluble fraction, each sugar constituting less than 0.1 per cent of the spore weight. Eight compounds corresponding in R_F values to glucose, galactose, mannose, arabinose, ribose, xylose, maltose, and fructose

were present and, in addition, two unknown NH_2–silver-nitrate reducing compounds. Analyses of the ethanol-soluble fraction also revealed the presence of cystine, aspartic acid, glutamic acid, serine, glycine, asparagine, threonine, alanine, glutamine, gamma-amino butyric acid, arginine, lysine, histidine, valine, methionine, leucine and/or isoleucine, phenylalanine, tyrosine, and eleven other ninhydrin-reacting compounds not yet identified. These unknown compounds broke down upon hydrolysis and other amino acids, proline and homocystine, appeared, indicating that the unknowns may have been soluble proteins, peptides, or amines. No additional amino acids were identified in the hydrolyzed ethanol-insoluble fraction. Qualitative and quantitative differences in sugar and amino acid composition were found among the races.

Observations on Pythium root rot of barley and wheat. BRUEHL, GEORGE W. Pythium root rot (*Pythium arrhenomanes* Drechsler) occurs in severe form as an endemic disease of cereals and grasses on a local soil of south-central South Dakota. This soil is low in available nitrogen. Addition of nitrogen in reasonable amounts to susceptible crops resulted in increased yields, not acting to further predispose the suscepts to greater losses. Field and greenhouse experiments indicate important varietal relations in barley. These relationships are influenced by two factors, one arising from individual inherent differences in susceptibility and the other associated with relative maturity classes. Very early varieties as a group are more susceptible than the later sorts. These varietal observations are based on mature plant reactions.

Nematode diseases and their control in mushroom crops. CAIRNS, ELDON J. That nematodes can cause considerable losses in commercial mushroom crops is now generally recognized by growers in the United States and abroad. These investigations show that species of *Ditylenchus*, *Aphelenchus*, and *Aphelenchoides* feed upon, injure, and destroy mushroom hyphae. Saprophytic forms (*Rhabditis* spp.) are often present in enormous numbers accompanied by an increased bacterial population. Experiments indicate that conditions unfavorable for mushroom mycelia are produced in the substrate. Saprophytic nematodes can also carry spot-producing organisms onto the fruiting bodies. The sources of nematode infestation are the manure compost, the mushroom house and bed structures, and the casing soil. The best control developed to date is the use of heat to kill the nematodes. Between crops, house and bed structures are water-soaked for 24-48 hr. to activate dormant nematodes, then disinfested by use of steam or steam plus formaldehyde. The air temperature is raised to at least 140° F. and held for several hours. Compost is treated in the beds by adding steam heat to raise pasteurizing temperatures, and using fans for better heat distribution. Casing soils are heated by steaming to above 150° F. for 15-20 min. or by use of soil fumigants in special soil bins.

Further studies on transmission of ringspot and cherry yellows viruses through seeds. CATION, DONALD. Seeds collected from two different Mahaleb trees infected with cherry yellows were germinated and grown to seedling stage in the greenhouse. They were later indexed on peach in the field. Seedlings from one tree showed 41 per cent total virus transmission and 24 per cent for the other tree. Each series showed a 2 to 1 ratio of cherry yellows to ringspot. A commercial source of Mahaleb seedlings indexed 11 per cent virus-infected, with a cherry yellows-ringspot ratio of 1 to 2. Montmorency seedlings produced from a cherry yellows tree showed 46 per cent infection with a cherry yellows-ringspot ratio of 1 to 4. Mazzard seedlings produced from a ringspot-infected Mazzard tree were 56 per cent infected with ringspot. There were indications that the viruses were not uniformly distributed through Mahaleb buds, some buds being apparently virus-free. There was a more uniform distribution through Mazzard buds. In a comparative test cherry trees propagated from disease-free buds on a commercial source of Mazzard stock showed 20 per cent cherry yellows symptoms in the field while 1.24 per cent of the trees similarly propagated on a commercial source of Mahaleb stock showed cherry yellows symptoms.

Dwarf fruit and decline of apple, a virus disease. CATION, DONALD, AND ROY E. GIBSON. By coincidence of an extensive grafting operation in two Hyslop crabapple orchards a virus present in some sources of the Jonathan apple variety was disclosed. Jonathan is a masked carrier. Hyslop proved a reactive indicator of the virus. The virus-carrying Jonathan scions grow normally for several years; later there is reduced growth and small-sized but normally shaped fruit. The entire tree gradually declines until the tree is dead or worthless in 5 or 6 years. Fruit borne on the Hyslop stock branches is dwarfed and deeply lobed or prominently five-ribbed longitudinally. The calyx end is characteristically oblate and the basin very shallow. Indications are that the virus entity transmitted by graft inoculations from diseased Hyslop to normal Hyslop is not so potent as that transmitted by Jonathan. The trees show less decline but the fruit distortion remains prominent. Several other sources of Jonathan produce normal fruit and cause no reactions when grafted to Hyslop stock.

Control of bacterial wilt of tomatoes by Bacillus polymyxa. CELINO, MARTIN S., AND DAVID GOTTLIEB. Attempts were made to control bacterial wilt of tomato by adding antagonistic microorganisms to soil. One isolate, B3A, a bacterium obtained from Philippine soil, appears to give significant control when added to soil infested with *Bacterium solanacearum*. This antagonist inhibits the growth of the pathogen on Petri plates. Furthermore, it can be produced when the bacterium is grown in a liquid medium, and gives large inhibition zones on assay plates. Soil which had been previously infested with the pathogen was treated by adding a broth culture of isolate B3A. After 19 days, Bonny Best tomato seedlings were planted in both the pots which had received the antagonist and the control pots which contained only the pathogen. A marked decrease of the disease was apparent in the treated soil; the controls had 79 per cent wilted plants, whereas the pots that had the antagonist had only 33 per cent wilt. Isolate B3A has been tentatively identified as *Bacillus polymyxa*. This organism is known to produce an antibiotic, polymyxin.

Variability among isolates of Pseudomonas tabaci. CHAMBERLAIN, D. W. The pathogenicity of six isolates of *Pseudomonas tabaci* was compared on soybean and tobacco. Three of the isolates were originally obtained from soybean, and three from tobacco. Greenhouse inoculations, wherein isolates were compared on opposite halves of the same leaf, showed tobacco isolates to be significantly higher in pathogenicity for tobacco than were the soybean isolates. On soybean the reverse was true, but differences were smaller. The pathogenicity of soybean isolates on soybean was extremely variable through five experiments, whereas tobacco isolates were uniformly high in pathogenicity for tobacco. All six isolates showed some degree of pathogenicity for both host plants. When soybean leaves, previously infected with bacterial pustule or bacterial blight, were inoculated with *Ps. tabaci*, a marked increase in wildfire infection occurred over inoculations where wounds or water-soaking provided the avenues of entry. *Ps. tabaci* appears to be less virulent as a primary parasite on soybean than on tobacco. The marked increase in wildfire infection in the presence of bacterial pustule and blight offers an explanation for the severe wildfire infection often found on field soybeans and the frequent failure of experimental inoculations.

Studies on the effect of environmental factors on the concentration of cucumber virus 1 in Nobel spinach. CHEO, PEN-CHING, AND GLENN S. POUND. The effects of several environmental factors on the concentration of cucumber virus 1 in Nobel spinach were studied by using the local lesion assay on cowpea. At air temperatures of 16°, 20°, 24°, and 28° C. there was at each temperature a cycle of virus activity. At each temperature, the virus concentration steadily increased for a time and then declined. The highest concentration obtained at any temperature depended upon the time interval after inoculation. Growing plants

at the specific temperatures prior to inoculation did not alter the cycle. Virus concentration was greater at a soil temperature of 28° than at 16°, with host vigor as a limiting factor. Increasing the day length from 8 to 16 hr. and the light intensity from 400 to 1600 foot-candles increased the virus concentration. In host nutrition studies with nitrogen, potassium, and balanced solutions, the virus concentration appeared to be directly related to vegetative growth. However, virus concentration increased with increased phosphorus even though the highest level caused stunting.

A species of Volutella parasitic on alfalfa. CHILTON, JOHN E. An apparently undescribed species of Volutella has been found causing leaf and stem infections of alfalfa. This species was first encountered on accessions in the Primary Plant Introduction Station greenhouse in Ames, Iowa. The cortex of petioles is invaded both inter- and intracellularly, killing the entire leaf. Linear stem lesions occasionally appear, at first brown, then becoming gray. The sporodochia are pink to white, erumpent, 40–70 microns broad, 50–80 microns long, with sparse, brown, continuous to 3-septate, marginal setae, 60–110 × 4–6 microns. The conidia are hyaline, mostly navicular, often biguttulate, 5.2–10 (7.5) × 2–3.5 microns, borne on simple conidiophores. Isolations from field plants included a variant producing identical conidia but having less prominent but more densely setose sporodochia. Both forms produce an appressed pink mycelial mat on artificial media, with conidia appearing in scattered sporodochial masses. The former type is more common in the field and has been found on *Medicago sativa, M. falcata, Trifolium pratense,* and *T. hybridum.* The fungus was also found at the Nebraska Agricultural Experiment Station on alfalfa plants grown from the same seed lots as were planted at Ames.

Testing methods for the evaluation of combinations of disease resistance in tobacco. CLAYTON, E. E., AND J. J. GROSSO. Tobacco breeding programs dealing with multiple resistance are being facilitated by testing procedures that permit critical evaluation of resistance to each disease, coupled with normal field growth and the opportunity for type selection. The test populations of plants are exposed successively to the diseases under study. However, each disease is fitted into the schedule at a point where it is possible to provide both the plant and the environmental conditions most favorable for the development of the particular disease. A typical schedule follows: Some 3000 seedlings from 151 segregating lines were (1) transplanted into soil infested with black root rot (*Thielaviopsis basicola*) in the greenhouse; (2) the root-rot-immune plants were then heavily shaded and inoculated with blue mold (*Peronospora tabacina*); (3) survivors were set into the field and sprayed with bacteria of wildfire (*Pseudomonas tabaci*) after moist, humid nights; (4) a few weeks later all were inoculated with common tobacco mosaic; and finally (5), late in the summer, all were subjected to a heavy natural infection by brown spot (*Alternaria longipes*). Several sets of susceptible checks were required, and at each step these were uniformly and severely affected by the disease under test. The period covered was 5 months of greenhouse and field culture.

The respiratory metabolism of Streptomyces scabies. COCHRANE, VINCENT W., AND HARRY D. PECK, JR. Oxidation of glucose-1-C^{14} and glucose-3,4-C^{14} by washed cells of *S. scabies* reveals a pattern different from that of related species and more consistent with the classical scheme of glycolysis. The endogenous CO_2 output of C^{14}-labeled cells is not appreciably reduced by concurrent oxidation of exogenous glucose, pyruvate, or acetate. Acetate-2-C^{14} as sole substrate is oxidized without dilution of specific activity; in the presence of glucose, on the contrary, significant dilution occurs, indicating the participation of acetate in glucose metabolism. Incorporation of C^{14} from acetate-2-C^{14} into *alpha*-ketoglutarate is demonstrated, although at a low level. Whole cells oxidize glucose, succinate, fumarate, malate, pyruvate, *alpha*-ketoglutarate, alcohol, and acetate, but not citrate or lactate. A cell-free preparation is shown to oxidize citrate in a single step to *alpha*-ketoglutarate, with quantitative yield of the latter. Malate is oxidized quantitatively to pyruvate by the same cell-free preparation. Oxidations by whole cells are affected strongly by age of the cells used; this is believed to reflect permeability differences. In general, the respiratory pattern is consistent with the operation of a tricarboxylic acid cycle.

Relation of the black rot pathogen to cabbage seed. COOK, A. A. Cabbage plants infected during the vegetative period are frequently symptomless until the flowering stage. By the time symptoms appear, the pathogen (*Xanthomonas campestris* (Pam.) Dows.) has become well established in the xylem of rachides, pedicels, and pods. The funiculi, which often dry down and remain attached to the seeds following threshing, are also often infected. The infection presumably may continue into the xylem of the seed coat from the funiculus, but this was not demonstrated histologically. Only rarely can the organism be recovered by plating of germinable seeds from infected pods. Use of the plating method as a bioassay of black rot infestation of seed lots is, therefore, unreliable.

Control of snow mold of forage crops in Alberta. CORMACK, M. W. Many cultivated and native plants proved susceptible to the low-temperature basidiomycete which often causes severe winter crown rot or snow-mold damage in alfalfa, clovers, and grasses in the central and northern areas. All varieties of alfalfa and clovers tested were susceptible except *Medicago falcata* and Siberian red clover. They are being used in breeding resistant varieties. Brome grass and *Agropyron* spp. were highly resistant and helped in maintaining mixtures of grasses and legumes. These grasses and spring-sown cereals or other annual crops were safely sown following severe damage. The pathogen did not persist in the soil to any extent for longer than 2 years in the absence of a susceptible host. Fertilizer treatments did not give beneficial results. Spraying the plants with a boron solution in the late fall markedly reduced the damage in some experiments, but there was a low margin of safety from boron injury. Mercuric chloride gave the best results of the many soil fungicides tested. Spread of infection was favored by the presence of uncut plant tops, or other debris, and by a slowly melting snow cover.

Resistance in safflower to root rot and rust in Alberta. CORMACK, M. W., AND F. R. HARPER. Root rot and rust have caused severe damage to certain varieties of safflower in experimental plantings at Lethbridge. Root rot, caused by *Pythium* sp., has occurred for several years in different locations on irrigated land but it has not been found on dry land. The symptoms are similar to those ascribed to *Phytophthora* spp. in other areas. Varieties and selections studied for 3 years ranked as follows: highly resistant—Indian, and Nebraska selections N3, N5, and N8; moderately susceptible—N6, N805; highly susceptible—N9, N10, N852. N3 has also shown some resistance to *Puccinia carthami* but the other root rot resistant varieties have proved highly susceptible to rust.

A toothpick tip method of inoculation. CRALL, J. M. The toothpick method of inoculating cornstalks was modified for studies of charcoal rot (*Macrophomina phaseoli*) and Diaporthe stem canker (*Diaporthe phaseolorum* var. *batatatis*) of soybean. The tips (1 cm. or less) of quill-type wooden toothpicks were boiled in several changes of tap water and pressed into the agar at the periphery of a vigorously growing fungus culture in a Petri dish. After the tips were covered with mycelium, they were inserted either into stem punctures (made with a steel probe) or into stubs of petioles cut off 15–20 mm. from the stem. The wounds were covered with petrolatum. Approximately 75 per cent of soybean plants thus inoculated developed typical charcoal rot symptoms; plants inoculated with mycelial suspensions or mycelium in agar were not infected. The percentages of stem canker-infected plants and plants with well developed cankers were as follows: stem (petrolatum), 100, 81; petiole stub (petrolatum), 68, 42; petiole stub (no petrolatum), 66, 1. Reliability of the method is high. The method is especially adapted to studies of weak patho-

gens and pathogens requiring conditions for establishment of infection that cannot be produced readily.

Studies on the control of white tip of rice. CRALLEY, E. M., AND R. G. FRENCH. The nematode *Aphelenchoides oryzae* Yokoo, the cause of white tip of rice, is carried over primarily in the seed. Evidence to date indicates that the nematode is not carried over in the soil from one crop season to another. Studies on the longevity of the nematodes in rice seed showed that where 8-, 20-, and 32-months-old seed was planted, white tip severity decreased as the age of the rice seed increased. Significant decreases in the severity of the disease have been obtained with the following seed treatments: (1) Parathion dust (25 per cent) and Systox on Carbon dust (50 per cent) at the rate of 2 oz. per bushel; (2) 12-hr. soak in a 1:1000 aqueous solution of mercury bichloride; (3) methyl bromide fumigation at the rate of 1½ lb. per 1000 cu. ft. of space; and (4) Aagrano dust at the rate of 2 oz. per bu. Methyl bromide fumigation slightly retarded seed germination when the moisture content of the rice seed was above 13 per cent. Aagrano dust and mercury bichloride soak treatments caused some mercurial toxicity.

Comparison of two nematocides used at the time of planting a winter cover crop. CRITTENDEN, H. W. As fall treatments with nematocides have been found to have certain advantages over spring treatments, it was of importance to determine whether treatment could be made at time of seeding of the winter cover crop. In a field test, three rates of ethylene dibromide (16, 26, and 36 gal./A.) and of the dichloropropene-dichloropropane mixture (13, 20, and 27 gal./A.) were used. Applications were made 14 days after seeding of rye and ryegrass. The reduction in stand by the ethylene dibromide for the three rates was respectively 12, 9, and 14 per cent under that of the untreated areas. The reduction in stand for the three rates of the dichloropropene-dichloropropane mixture was respectively 38, 33, and 53 per cent. In the greenhouse tests, applications of the two compounds at the three rates were made in three types of soil—loam, sandy loam, and sand—at the time rye was planted. Data in all treatments showed that ethylene dibromide was less toxic than dichloropropene-dichloropropane. The evidence indicates that ethylene dibromide is preferable if application must be made at or close to the time of planting of a rye cover crop.

Resistance of asparagus to Meloidogyne incognita var. acrita. CRITTENDEN, H. W. In published literature, *Asparagus officinalis* is reported to be heavily infested by the root-knot nematodes; however, there are only four references and no exact data are presented on the degree of infestation. From preliminary greenhouse work done in 1950, with the Mary Washington variety grown in soil heavily infested with *Meloidogyne incognita* var. *acrita*, it was determined that there was no hypertrophy of the roots. During the summer of 1951, asparagus that was planted in a field heavily infested with this species of nematode was examined periodically. After 5 months' growth, the roots showed no hypertrophy. Microscopic examination showed the average occurrence of females with egg sacs to be one per plant. Cantaloupes adjacent to the asparagus were heavily knotted. These observations indicate that asparagus would be a suitable crop to use on land infested with *Meloidogyne incognita* var. *acrita*.

Macroscopic growth of the oak wilt fungus in nature. CURL, E. A., G. J. STESSEL, AND BERT M. ZUCKERMAN. During August and September, 1951, *Chalara quercina* was found as large, macroscopically visible mycelial mats between the bark and wood of diseased oaks in widely separated forests in Illinois. Growth of the fungus as seen in the field, and its sporulation, closely resembled the growth and sporulation that take place on artificial media. Pure cultures usually could be obtained by direct transfer of the mycelium. The fungus was observed in the cambial region and frequently grew along insect galleries occupied by adults and larvae belonging to three insect families. The trees on which growth of the oak wilt fungus was found had been invaded by wood and bark borers of several species; the bark was fairly loose. From one tree, when it was felled, samples were taken that showed sparse growth of a tan fungus between wood and bark. This fungus developed into large sporulating mats of *C. quercina* in 48 hr., while the samples were kept in a moist, closed box. On similar samples that were discarded when cut because they bore no evidence of fungus growth, sporulating mats of the oak wilt fungus developed within a week as they lay on the forest floor under felled logs.

Chemotherapeutic activity may be independent of fungitoxicity. DAVIS, DAVID. A comparison of the chemotherapeutic activity of a number of compounds against the Fusarium wilt disease of tomato and their fungitoxicity to the pathogen indicated no consistent relationship. Chemotherapeutic activity was determined by the method described by A. E. Dimond and R. A. Chapman (Phytopath. 41:11. 1951). Fungitoxicity was determined by *in vitro* growth and respiratory responses of mycelium of *Fusarium lycopersici*. Both 120 micromolar sodium 2-carboxymethylmercaptobenzothiazole and 410 micromolar 2-(n-amyl) pyridine had high chemotherapeutic activity, but only partially depressed mycelial growth at 5,000 micromoles per liter. The 75 micromolar 4-chloro-3,5-dimethylphenoxyethanol and 86 micromolar n-octadecyltrimethylammonium pentachlorophenate were highly chemotherapeutic, and reduced growth at equivalent concentrations. A 2000 micromolar oxyquinoline benzoate had no chemotherapeutic activity, but 100 micromoles per liter inhibited mycelial growth completely. Excepting oxyquinoline benzoate, inhibition by these compounds of oxygen uptake approximately paralleled their mycelial growth-inhibiting capacity. An aqueous extract of whole tomato plants grown for 6 days on 75 micromolar 4-chloro-3,5-dimethylphenoxyethanol was not fungitoxic when tested against spores of *Stemphylium sarcinaeforme*. However, macerated tissue from these plants exhibited approximately a 20 per cent increase in oxygen uptake and invert sugar content. 4-Chloro-3,5-dimethylphenoxyethanol may alter the susceptibility of the host to disease by causing pronounced changes in its metabolism.

Free amino-acids and carbohydrates synthesized by Ustilago zeae. DEVAY, J. E., J. B. ROWELL, AND E. C. STAKMAN. Chromatographic analyses showed that culture extracts from two compatible haploid lines and a solopathogenic line of *Ustilago zeae* contain glutamic acid, aspartic acid, serine, glycine, alanine, threonine, glutamine, lysine, arginine, gamma-amino butyric acid, proline, valine, leucine and/or isoleucine, phenylalanine, and tyrosine. An unknown ninhydrin-reacting compound, stable to hydrolysis, was found only in extracts from one haploid line. Another unknown was found only in extracts from the solopathogen. Quantitative variations among the amino-acids were found when analyses of the three lines were compared. Young cultures contained relatively large amounts of glutamic acid; in older cultures other amino-acids increased greatly. The presence of free amino-acids was affected by the degree of culture aeration and culture age. Changes in the sugar content of aging cultures were followed by sugar chromatography of the amino-acid extracts. Maltose, sucrose, fructose, ribose, and six unidentified NH_3-silver-nitrate-reducing compounds were found. Cellobiose, arabinose, xylose, and rhamnose corresponded in R_F values on one-dimensional chromatograms to four of the six unknowns.

Interaction between cucumber mosaic and Phytolacca decandra. DIACHUN, STEPHEN. Four isolates of cucumber mosaic virus from burley tobacco produced local necrotic and chlorotic spots and rings and systemic infection in *Phytolacca decandra*. The reaction of noninoculated leaves on plants inoculated with any one of these isolates ranged from severe distortion and blistering, mottling, necrosis, and dwarfing through mild chlorotic ring patterns to practical immunity. These isolates of cucumber mosaic were transferred readily from *Phytolacca* to *Phytolacca*, but less frequently from *Phytolacca* to tobacco or cucumber. Inoculum from *Phytolacca* produced numerous local lesions on rubbed leaves of susceptible *Phytolacca*, but very few or no lesions on rubbed leaves of susceptible cowpea. Addition of sap

from healthy leaves of *Phytolacca* to inoculum from tobacco inhibited local lesions on cowpea, but did not inhibit local lesions, or at least did so to a lesser degree, on *Phytolacca*. These results suggest that the inhibitor acts on the host rather than on the virus. Cucumber mosaic is not the cause of the common Phytolacca mosaic in Kentucky, nor of mosaic in two plants from Connecticut and one from Virginia.

A method for inducing abundant sporulation of Stemphylium solani in pure culture. DIENER, U. L. Copious production of conidia was obtained when cultures of *S. solani* growing on an agar medium containing 20 per cent of canned vegetable juices ("V-8," Campbell Soup Co.) were irradiated with ultraviolet light. Petri-dish cultures with the covers intact were exposed 15 cm. from the light source (Westinghouse Sterilamp WL-30). After a 5-min. irradiation a 5-day-old culture approximately 40 mm. in diameter growing at 28° C. produced 30,000 or more conidia in a zone near the periphery of the colony within 24 hr. Repeated irradiations at 15- to 18-hr. intervals resulted in the production of conidia in successive crops at the margin of the colony. Although sporulation was profuse on 20 per cent vegetable-juice medium, some conidia were produced on media in which the concentration varied from 1 to 64 per cent by volume. Abundant sporulation occurred over a pH range of 4.0 to 7.5, and a few conidia were produced without irradiation at pH 4.0 to 4.5.

2-Carboxymethylmercaptobenzothiazole salts as chemotherapeutants for plant diseases. DIMOND, A. E., AND DAVID DAVIS. Alkali salts of 2-carboxymethylmercaptobenzothiazole are not fungitoxic in spore germination tests nor are they changed to fungitoxic compounds by contact with living tissue. However, in controlled greenhouse experiments, soil applications of these compounds have markedly reduced the incidence of *Fusarium lycopersici* in the roots and of *Alternaria solani* on the leaves of treated tomato plants. In field experiments these compounds materially reduced the disease grade of *Verticillium* wilt of eggplant (8 per cent in treated versus 30 per cent in check plots) when applied five times at weekly intervals as a soil treatment to inoculated plants in heavily infested soil. They reduced the severity of Dutch elm disease when applied as a foliage spray with a non-phytotoxic oil. Such sprays were applied five times at weekly intervals and trees were then inoculated with *Ceratostomella ulmi*. Four weeks later the disease grades in the treated and control plots were, respectively, 6.2 and 32 per cent, while 8 weeks later they were 12 and 58 per cent. Spraying therapeutants on elms is much easier and cheaper than soil application and makes possible the treatment of many city trees that are surrounded with pavement.

A ringspot disease of peppers caused by strains of cucumber mosaic virus from pepper and alfalfa. DOOLITTLE, S. P., AND W. J. ZAUMEYER. Peppers in Maryland, Virginia, and Delaware occasionally have large yellow rings on leaves and fruits; leaves developing later are mildly mottled and slightly dwarfed. These symptoms have been reproduced by inoculation with a strain of cucumber mosaic virus (*Marmor cucumeris*) isolated from ringspotted peppers, and with a closely related strain of the same virus isolated from mottled alfalfa leaves collected in Idaho. This isolate originally was found combined with an alfalfa mosaic virus. On peppers, the complex from alfalfa caused symptoms similar to those produced by the cucumber virus strains. On tobacco, both strains caused pronounced necrotic ring and oak-leaf primary lesions and a mild systemic mottle. Cucumber seedlings had yellow asteroid spots on young leaves, but later symptoms resembled those of typical cucumber mosaic. On most other hosts tested, both isolates caused similar symptoms. Only the alfalfa strain infected the resistant Old Dominion spinach, but both strains infected Long Standing Bloomsdale, which is susceptible to the cucumber mosaic virus. Both caused systemic symptoms on several bean varieties and on white sweet clover, crimson clover, cowpea, and sweet pea. On zinnia, both protected against Price's No. 6 strain of cucumber mosaic virus.

Comparison of two methods of inoculation on the sporulation of 40 fungi. EICHENMULLER, J. J. The rate and amount of sporulation of 40 fungi were compared when two methods of inoculation were used: 1) flooding the agar surface of Petri dishes with a heavy suspension of spores, and 2) placing a loopful of spores on the center of the plate. All of the fungi tested produced spores more rapidly, or more abundantly, or both, when method 1 was used. The rate of sporulation increased directly as the numbers of spores increased in the suspension used for inoculation. This rapid rate of sporulation may be due to exhaustion of nutrients, particularly sugar, from the medium. High concentrations of sugar delayed sporulation. The rate of exhaustion of sugar from the medium was studied and it was found that sporulation began about the time the sugar was used up. The rates of respiration of several fungi inoculated by the spore-flooding technique were determined. Sporulation was initiated at or near the peak of the respiration curve.

The importance of the stylar region of the pepper fruit in the control of fruit disease. EVANS, MARSHALL, AND T. H. KING. In studies started in 1949, it was determined that an abnormally high percentage of fruit produced by various varieties of peppers had a definite hole in the stylar end of the fruit. Microorganisms are nearly always present inside such fruits. *Fusarium* sp., *Alternaria* sp., and bacteria are commonly isolated from the seed and affected tissue. In seven varieties examined in 1949 and 1950, the percentage of diseased fruit ranged from 9 to 52. By selecting seed from disease-free fruit it was possible to obtain progenies with fewer fruits having a hole in the stylar region. Thus it is possible to breed for resistance to internal fruit rot, by using the structure of the stylar region as a selective character.

Evaluation of eradicant fungicides against Ceratostomella fimbriata. FEAZELL, GEORGE D., AND W. J. MARTIN. The need for fungicides that could be used for rapid disinfestation of sweet potato washing machines which have become contaminated with *Ceratostomella fimbriata* prompted the testing of various chemicals for that purpose. Although various mercurials are known to be eradicant against *C. fimbriata*, their use on washing machines cannot be recommended because of their toxicity to human beings. In testing chemicals that possibly could be recommended, the following technique proved useful: sterile vegetable brushes were dipped in a spore suspension of the fungus, then placed in an aqueous solution or suspension of the chemical for 30 sec. The brushes were dipped in three successive changes of sterile water and then touched to the surface of potato-dextrose agar in Petri plates. Effectiveness of the chemicals was determined on the basis of subsequent mycelial growth of *C. fimbriata* on the potato-dextrose agar plates. Of the materials tested the most promising for the purpose were: Bioquin 1 (copper 8 quinolinolate), Santobrite (sodium pentachlorophenate), and Vancide 51 (sodium salts of dimethyl dithiocarbamic acid and 2-mercaptobenzothiazole). Solutions of copper sulfate were completely ineffective.

Organic vs. inorganic fungicides for the control of the brown-rot fungus on peaches. FOSTER, H. H. In 1951 ten spray treatments were compared in randomized single tree plots on the peach variety J. H. Hale. Eight applications of each fungicide were made with a power sprayer; the first spray was applied at petal-fall. Peaches were harvested approximately 2 weeks after the final fungicide application. In certain storage experiments the peaches were held at room temperature (approximately 75°–80° F.) for 5 days after harvest. In other experiments, after 5 days at approximately 45° F., peaches were ripened for 3 days at room temperature. Data were recorded after these 5- or 8-day storage periods. The fungicides are listed below in decreasing order of control of the brown rot fungus (*Monilinia fructicola*). Wettable sulfur (6 lb./100 gal.), Orthocide 406, manganese ethylene bis dithiocarbamate, wettable sulfur (4 lb./100 gal.), Dithane D-14 and ferric sulfate, General Chemical 1189, Rohm & Haas CR-2379, Parathion, dispersible sulfur, and calcium hypochlorite. Only the first

three mentioned appeared sufficiently effective for practical control of brown rot. Peaches sprayed with Orthocide usually had a better color than those sprayed with wettable sulfur.

Seven virus diseases of cucurbits in California. FREITAG, J. H. Seven viruses believed to be separate and distinct have been isolated from cucurbitaceous plants growing in California. These viruses are those of cucumber mosaic, squash mosaic, muskmelon mosaic (cucurbit ring mosaic), wild cucumber mosaic, cantaloupe mosaic, western watermelon mosaic, and muskmelon vein necrosis. The last two viruses have not been previously reported. In contrast to cucumber mosaic virus the other six viruses all have limited host ranges. Cantaloupe mosaic virus and watermelon mosaic virus are typical aphid-borne viruses and are efficiently transmitted by the melon and green-peach aphids during short feeding periods following a period of starvation. Squash mosaic, muskmelon mosaic, and wild cucumber mosaic viruses were demonstrated to be transmitted by beetles, but not by aphids. A new virus, that of muskmelon vein necrosis, has been transmitted in preliminary trials to several varieties of muskmelon. The disease is characterized by a necrosis of veinal tissue which results in drying of the leaves and produces irregular necrotic, corky areas along the stem. The seven viruses can be separated on the basis of symptoms, insect vectors, and physical properties.

Rhizoctonia leaf blight of strawberries. FULTON, J. P. In late summer of 1950 a severe leaf blight of strawberries was widespread in several areas in Arkansas. Black to dark brown lesions, often angular and irregular in outline, appeared on the leaves. Leaves were rapidly killed. Affected areas in the field were circular, 1–4 ft. in diameter. In these affected spots all of the leaves were killed but the crowns were not injured. The disease was most severe in 1-year-old fields and in areas where fertilizer treatments had promoted a heavy foliage. The fungus, *Rhizoctonia solani*, was consistently isolated from diseased material. Inoculation of healthy plants reproduced the symptoms observed in the field. Other isolates of *R. solani*, either from strawberry roots and crowns or other hosts, failed to produce similar symptoms. In a few cases leaf spotting of strawberries was obtained but it was limited in extent and readily distinguishable from that produced by the strawberry leaf isolate. The disease, as observed in 1950, did not severely damage strawberry plantings. The following spring there was very little evidence of any reduction in vigor or fruit production in affected areas.

Studies on Verticillium wilt of raspberry. FULTON, ROBERT H. Single conidial isolates yielded four biotypes, three of which were used to infect raspberry. S1 (microsclerotial) and S2 (fluffy mycelium) infected raspberry at 24° C., while S3 (appressed mycelium) infected at 28° C. The infection periods were shortened when the canes or roots were injured. Suckering was reduced in infected red raspberries. The toxin theory of wilting appeared most plausible. Filtrates from the three biotypes were not toxic unless the fungi were grown for at least 30 days in synthetic broth and exposed to continuous light. Biotype S1 was an exception. When grown in the dark, its filtrate became toxic following exposure to light for 30 hr. After ultrafiltration the nonfilterable portion produced a wilt without discoloration of the vessels while the filtrate blackened the vessels without wilt production. This indicated that the toxin may be a complex of several compounds varying in particle size. In biotype S2 there were two optimum growth reactions to acidity, pH 5.5 and pH 8.0. The biotypes changed acid media to an alkaline state aproaching their requirements for optimum growth.

Comparison of fungicides for the control of spur blight and anthracnose of raspberry. FULTON, ROBERT H., AND JOHN P. TOMPKINS. Sprays for spur blight control were applied to heavily infected Latham when the suckers were 7–10 in. high, and again 12 days later. On unsprayed plants 60 per cent of the buds were infected. Ferbam resulted in 10.4 per cent, ziram 25.6 per cent, and Tribasic copper 32.5 per cent infection; other materials resulted in the following infection counts: actidione, 35.7 per cent; manganese ethylene bis-dithiocarbamate, 36.5 per cent; Ortho 406, 38.0 per cent; nabam, 39 per cent; zineb, 40.2 per cent; Phygon XL, 43.4 per cent; and Crag 341, 44.8 per cent. The 1 per cent Krenite 26 (sodium salt of dinitro ortho cresol) applied as a dormant spray reduced the infection only 10 per cent. A spreader, Triton B 1956, increased the effectiveness of sprays 6 per cent. Anthracnose on unsprayed Cumberland's averaged 173 lesions for a single cane. A dormant application of 1 per cent Krenite 26 reduced this infection 55.5 per cent; a delayed dormant spray of liquid lime sulfur (12½ gal./100 gal.) gave 68 per cent reduction. Bordeaux 6-12-100 plus 2 qt. Dendrol as a delayed dormant reduced infection 32 per cent. For summer applications following either Krenite or lime sulfur dormant sprays, both ferbam and Ortho 406 gave comparative control, reducing infection approximately 80 per cent for one application and 93 per cent for two applications. Nabam and manganese ethylene bis-dithiocarbamate in cover sprays did not give satisfactory control.

Mosaic resistance in Wisconsin tobacco. FULTON, ROBERT W., AND JAMES JOHNSON. The necrotic local lesion type of resistance (N factor) to tobacco mosaic virus has been incorporated in tobacco closely resembling Havana Seed types. Lines derived from crosses of Wisconsin tobacco with mosaic-resistant Burley have more closely resembled Wisconsin types than have lines from crosses with dark tobaccos. Segregation in the F_2 generation for mosaic resistance and chlorophyll characters were close to the ratios expected on the basis of independent factors. Selected F_5 lines from the original cross are apparently of as good or better commercial type, quality, and yield as lines which have been backcrossed four times to the commercial parent. Systemic necrosis as a result of heavy virus inoculation developed in appreciable amounts only during the hot summer of 1949. Different lines varied from 0 to 48 per cent of the plants systemically necrotic. Several otherwise desirable lines are susceptible to a late-season leaf-spotting which is absent or minor on other lines and on Havana Seed. Commercial trials on a small scale have demonstrated very satisfactory mosaic resistance and commercial acceptability. Infection of mosaic-resistant tobacco by cucumber mosaic virus has occurred only rarely the past two seasons.

Development of Erysiphe graminis tritici on susceptible and resistant common spring wheats. FUTRELL, MAURICE C. Penetration and subsequent development of *Erysiphe graminis tritici* on seedling leaves of susceptible (Henry C. I. 12265) and resistant (C. I. 12633, a *Triticum timopheevi* derivative) wheats grown in the greenhouse at 16° C. were studied cytologically. Conidia germinated in 12 hr. on inoculated leaves of Henry, and penetration of the epidermal cells was completed within 72 hr. Germ tubes gave rise to small appressoria that produced thin penetration hyphae. Changes in staining reaction occurred in the cell wall immediately after penetration of the cuticle. Even though cell walls thickened on the inside, the hyphae penetrated rapidly and haustoria were established readily. Surface mycelium developed profusely and sporulated abundantly; under certain greenhouse conditions perithecia appeared in 35 days. In C.I. 12633 the cuticle and cell wall were penetrated in like manner, but hyphal development was stopped at contact with host cytoplasm. The cytoplasm of epidermal cells appeared disorganized soon after contact with the hyphae and infected cells soon collapsed. Prior to or simultaneously with collapse of epidermal cells the adjacent mesophyll cells showed abnormal staining. Macroscopic flecks appeared 4 or 5 days after inoculation.

Production of galls in Kalanchoe by variants of Agrobacterium tumefaciens differing in colonial morphology. GAINOR, C., AND W. C. PRICE. From a highly virulent single colony derived by the serial streak method from A.C. Braun's B6 strain of *Agrobacterium tumefaciens*, three colonial variants have been obtained and maintained in pure culture for more than 5 months of continuous subtransferring. The variants are differentiated on the basis

of colonial size and appearance, the latter characteristic ranging from smooth to lobate and radially striated. No significant differences in biochemical reactions of the three isolates have been found, all three showing reactions essentially the same as those reported by Sagen, Riker, and Baldwin in 1934. Agar-plate cultures of each variant 2, 4, and 8 days old produced typical galls in *Kalanchoe daigremontiana*. Moreover, galls 30 days old were found to contain an essentially pure culture of the variant used to induce the infection.

Variability in the onion pink root fungus induced by nitrogen mustard. GASIORKIEWICZ, E. C. Treatment of spores from two natural isolates of the onion pink root organism, *Pyrenochaeta terrestris* (Hansen) Gorenz et al., with methyl-bis (β-chloroethyl) amine induced cultural and pathogenic variability. There was no correlation between the type of mutants produced and the duration of treatment. Pathogenicity tests wherein 12 mutants in the Louisiana series and 13 in the Wisconsin series were tested on two susceptible and two resistant onion varieties showed marked differences in disease indices as well as in pycnidium production. Through increased or decreased virulence some of the mutants produced a disease comparable to that ascribed to some of the natural isolates. Sporulation on the host was obtained in greenhouse as well as in field tests, but was not correlated with pathogenicity. Sparsely sporulating mutants were as virulent as the sporulating types. Mycelial lines varied from very low to moderately high disease indices. Most of the induced mutants were found to fall within the descriptive and cultural range of the natural isolates. Cytological evidence of uninucleate spores supports the possibility that new and more virulent strains of *P. terrestris* can be obtained by mutation of existing natural strains.

Some apparent virus relationships in several orchid genera, based on electron microscopy. GOLD, A. HERBERT, AND D. D. JENSEN. An electron-microscope study of virus particles isolated from plants of several orchid genera indicates the prevalence of two general particle shapes. One of these is a sinuous rod about 480 mu × 18 mu. The other is a sickle-shaped rod about 300 mu × 28 mu. The sinuous rod has been isolated from diseased plants of *Cymbidium*, *Cattleya*, *Oncidium*, and *Laelia*. The sickle-shaped rod has been isolated from *Cymbidium*, *Cattleya*, and *Odontoglossum*, and from *Zinnia* which had been juice-inoculated from *Odontoglossum*. Certain leaf symptoms of naturally infected Cymbidium plants are distinctively associated with each type of particle. Mixtures of both types of particles frequently occur in natural infections of *Cattleya*, though individual plants may show only one type of particle. The presence of either type of particle appears to be associated with abnormal pigment patterns in the flowers of *Cattleya*. No particles of either type have been recovered from healthy seedlings. When inoculum containing only one type of virus particle was used on Cymbidium seedlings, that type of particle was recovered from these seedlings. Odontoglossum seedlings developed symptoms and contained particles similar to those in the inoculum only when inoculated with inocula containing the sickle-shaped particles.

The disappearance of antibiotics from soil. GOTTLIEB, DAVID. To determine whether antibiotics take part in the antagonism of microorganisms toward each other in soil, one must determine whether such materials persist under these conditions. In normal soils the diverse microflora degrades many antibiotics; chloromycetin is stable in sterile soil but is rapidly inactivated in nonsterile soil. At the high concentrations of 50 μgm. per gm. of soil there was an initial 24-hr. lag period followed by a rapid decline to 43 per cent at 72 hr. and to 15 per cent after 2 weeks. The degradation is fairly complete, for no inactive aryl nitro compounds or aryl amines could be detected in the soil. Similar studies with clavacin indicate that the microflora of soil will rapidly inactivate this compound. At 48 hr. no antibiotic could be detected in concentrations above the sensitivity of the assay. In the sterile control soils the concentration of clavacin was not reduced even after 7 days. Actidione also is unstable in soil; within 11 days' incubation 92 per cent of the actidione could not be recovered from nonsterile soil and 53 per cent was unaccounted for in sterile soil. Basic antibiotics such as streptomycin and streptothricin are inactivated by the clay, the organic matter, and the microflora of soil.

A Fusarial disease of seedling asparagus in Ontario. GRAHAM, K. M. Symptoms of the disease, observed for the first time in Canada in 1945, were stunting, yellowing, wilting, and pre-emergence blight. Penetration of the pathogen was appressorial and usually occurred in the embryonic region of the root tip. Colonization of the host was limited to the cortex. Surveys indicated a wide distribution of the pathogen in the Niagara Peninsula, on soils used for asparagus culture. The pathogen was identified as a strain of *Fusarium oxysporum* Schlecht. On potato-dextrose agar it was overgrown by a mycelial type which proved to be less pathogenic than the sporulating type. Macroconidia were produced readily on soil agar and water agar. The optima for growth of the pathogen, growth of the host, and for disease incidence were between 25° C. and 30° C. Best emergence and development took place at a medium soil moisture. The growing and incorporation, in naturally infested soil in flats, of three successive crops of soybeans, of oats, and of potatoes resulted in an increase in disease incidence over that following three crops of asparagus. The manner of placement in the seed bed of a soil disinfectant, Arasan (3 gm. per sq. ft.), was found significant in controlling the amount of pre- and post-emergence damping-off. Nine cultivated varieties and strains of *Asparagus officinalis* L. were susceptible.

Nematodes as ectoparasites on tobacco, cotton, and other plants. GRAHAM, T. W. Examination of soil screenings from the root area of diseased tobacco, cotton, corn, and other plants indicated that ectoparasitic nematodes are important parasites on these crops in the Southeast. Christie's soil screening technic was used to recover nematodes. Field collections of soil from diseased cotton, corn, cowpeas, soybeans, and strawberries yielded high populations of the sting nematode, *Belonolaimus* sp. Similar collections from tobacco and cotton gave large numbers of *Tylenchorhynchus* sp. *Rotylenchus* sp. was dominant in collections from diseased cotton, gardenia, and other plants. *Trichodorus* sp. was found associated with corn, cotton, and various grasses. Separate soil cultures of these nematodes were established in the greenhouse. Singly picked nematodes or mass transfer of a single parasitic species were added to pots of steamed soil. Cotton, tobacco, and tomato grown in Rotylenchus cultures were stunted and had root decay. Corn and cowpeas in Belonolaimus cultures were severely diseased, but tobacco was not affected. Tobacco in Tylenchorhynchus cultures was diseased as were corn and cowpeas in Trichodorus cultures. In most cases nematodes increased in large numbers, field symptoms were reproduced, and inoculated species remained free of other parasitic nematodes. Plants in control pots grew normally.

Carrot root scab—a disease complex. GROGAN, R. G., AND JAMES B. KENDRICK, SR. Root scab showing various types of symptoms seriously affects carrot production in the central coastal section of California. In some cases the lesions are black and sunken and usually occur in root scars. *Stemphylium radicinum* (M. D. & E.) Neerg. causes this type of lesion. In other cases the lesions are reddish-brown and raised, and usually are located in the area between the lines of lateral roots. Isolations from different types of lesions have yielded a variety of fungus and bacterial organisms which have proven nonpathogenic in greenhouse tests. The reddish-brown, raised lesions occur on roots grown in steam-sterilized soil from treated seed and are believed to be nonpathogenic in origin. They are often closely associated with oil ducts and oil-filled, unbroken blisters on the roots. Individual roots show wide variation in their propensity to develop this type of symptom. Often one of two roots growing side by side and touching will have severe symptoms, the other no symptoms. It appears

that this type of lesion results from abnormal oil production resulting from the interaction of a certain genotype with climatic factors peculiar to this area.

Cross-protection tests with viruses affecting canning peas in Wisconsin. HAGEDORN, D. J. A series of cross-protection tests was conducted in the greenhouse with five viruses which originally had been isolated from canning peas in Wisconsin: bean virus 2, cucumber mosaic virus strain 28, pea enation mosaic virus, Wisconsin pea streak virus, and the red clover vein-mosaic virus. Appropriate leguminous test plants, which characteristically showed differential symptoms, were used. These included crimson clover, fenugreek, pea, red clover, and soybean. In most cases each virus was tested against each of the other four viruses in at least two separate trials. There were no results which conclusively showed that one of these viruses could protect the plant from infection by any of the other viruses. Thus, through the use of cross-protection tests, there is added evidence that these viruses are seemingly unrelated.

Experiments with pea seed protectants in Wisconsin. HAGEDORN, D. J. Canning pea seed protectants were compared in experiments at Madison, Wisconsin, during 1949, 1950, and 1951. The following seed protectants were studied: Arasan, Arasan SF (slurry), Dow 9B, KF467, Phygon, Phygon XL (slurry), Phygon Seed Protectant (slurry), Spergon, and Spergon SL (slurry). Properly randomized and replicated studies were made in greenhouse temperature tanks at 16° C., in a walk-in refrigerator at 12° C., and in the field. Different soil types were used and trials were repeated in most cases. Appropriate controls were included. The 1949 and 1950 results, whether from temperature tanks, refrigerator, or field, indicated that Arasan, KF467, Phygon, and Spergon were significantly more effective than Arasan SF, Dow 9B, or Phygon XL. Higher dosages, especially for slurry treatments, were used in 1951 and results of temperature tank and field experiments showed that KF467 and Phygon Seed Protectant were best. It was observed that reliable comparisons of pea seed protectants could be made in either greenhouse temperature tanks or walk-in refrigerator, or in the field.

Relative pathogenicity of seven species of Pythium on red clover seedlings. HALPIN, J. E., E. W. HANSON, AND J. G. DICKSON. *Pythium debaryanum* Hesse, *P. irregulare* Buisman, *P. splendens* Braun. *P. ultimum* Trow., *P. paroecandrum* Drechsler, *P. arrhenomanes* Drechsler, and *P. rostratum* Butler were tested in the greenhouse at 16°, 20°, 24°, and 28° C. to determine their relative pathogenicity on red clover seedlings. The seedlings were grown for 15 days in glass tumblers containing a mixture of autoclaved white silica sand, sterile 1 normal Hoagland solution, a small amount of agar, and the fungus to be tested. All tests included checks and were repeated three times using four replications at each temperature for each trial. Six species were pathogenic; *P. rostratum* was not. *P. debaryanum* was most pathogenic followed by *P. irregulare*, *P. splendens*, *P. ultimum*, *P. paroecandrum*, and *P. arrhenomanes* in descending order. Pre-emergence killing, post-emergence damping-off, root rotting, spotting of the cotyledons and hypocotyls, and stunting were common symptoms. In general, root rotting involved a necrosis of the cortical areas but more advanced stages resulted in complete destruction of the root system. Pathogenic species caused disease over the entire temperature range of 16° to 28° C. but usually were more pathogenic at the two higher temperatures.

Panogen and Vancide 51 compared with standard seed treatments for control of wheat, oat, and sorghum smut in Kansas. HANSING, E. D. Red Chief wheat artificially infected and planted in the field produced 57 to 95 per cent bunt. Seed treated with Panogen (1.4, 1.7, 2.0, and 2.3 per cent mercury) at combinations of ½, ¾, 1, 1¼ and 1½; New Improved Ceresan at ½ and 1; Ceresan M at ½ and 1; Vancide 51 at 4 and 6; copper carbonate at 3; and Spergon at 3 oz./bu. and planted immediately and 1, 2, 4, 8, and 16 days after treating, produced (except Panogen, ½ oz., immediately) crops with 1 per cent or less bunt. Concentration, rate, and time after treating were inversely correlated with infection for Panogen, New Improved Ceresan, and Ceresan M. For Vancide 51, infection increased with time after treating. Kanota oats artificially infected with loose smut (partial vacuum method) produced 53 to 75 per cent smut. Seed treated similarly with Panogen, New Improved Ceresan, and Ceresan M permitted 1 per cent or less smut when planted 4 to 16 days after treating. Similar but more definite correlations were obtained. Vancide 51 at 8 and 24; Arasan at 4; and Spergon at 4 oz./bu. were not satisfactory for control of oat smut. Red kafir artificially infected produced 37 and 52 per cent kernel smut. Panogen (1.4 per cent mercury) at ¾, 1, 1¼, and 1½; New Improved Ceresan at ½ and 1; Ceresan M at ½ and 1; Vancide 51 at 4 and 6; Arasan at 2; and Spergon at 2 oz./bu. gave complete control.

Panogen and Vancide 51 compared with standard seed treatments on control of Victoria blight of oats and emergence of wheat, oats, and sorghum in Kansas. HANSING, E. D. Osage oats artificially inoculated with *Helminthosporium victoriae* yielded 5 bu. per acre. Inoculated seed planted 2 and 14 days after treating yielded: Panogen, ¾ oz., 29; New Improved Ceresan, ½ oz., 28; Ceresan M, ½ oz., 22; Vancide 51, 12 oz., 9; and Spergon, 4 oz., 5 bu. per acre. Seed of wheat, oat, and sorghum varieties were treated with Panogen at ¾, 1, and 1½, New Improved Ceresan at ½ and 1, Ceresan M at ½ and 1, Vancide 51 at 2 (plus 2 water), 4, and 6, Arasan at 2, and Spergon at 2 oz. per bu. and planted in the field on two dates in 1950 and 1951. Vancide 51 at 4 and 6 oz. and Arasan produced greatest increases in emergence. Panogen, New Improved Ceresan, and Ceresan M gave comparable increases in stand. Higher rates of Panogen, New Improved Ceresan, and Ceresan M more effectively controlled Victoria blight and increased emergence of sorghum but decreased emergence of wheat and oats. Higher rates of Vancide 51 increased emergence of wheat, oats, and sorghum.

Factors associated with stem rot of tobacco caused by Botryosporium. HEGGESTAD, H. E., AND D. R. BOWMAN. Development of stem rot caused by *Botryosporium pulchrum* Cda. has been associated with prolonged moist curing conditions and abnormally hygroscopic tobacco having high chlorine content. The fungus is semiparasitic on partially cured tobacco. It is characterized by its white aerial mycelium and dense clusters of conidia found on leaf midribs and larger veins. Leaves of a severely affected 1947 crop averaged 5.15 per cent chlorine. When conditioned at 82 per cent relative humidity this tobacco contained 29.6 per cent moisture compared to only 16.8 per cent for leaves with 0.12 per cent chlorine. In 1949, the fungus was destructive on tobacco from manured plots but not on tobacco from plots in the same series grown without manure. Tobacco from the manured plots was more hygroscopic and higher in chlorine content. Considering leaf parts, the fungus caused maximum damage to the midribs and minimum damage to the lamina. Chemical analyses of midribs, veins, and lamina from a severely affected 1950 crop revealed 5.64, 4.13, and 2.65 per cent chlorine, respectively. When the latter samples were conditioned at either 82 per cent or 100 per cent r. h. the midribs and veins absorbed 5 to 6 per cent more moisture than the lamina.

A new species of Peridermium on Virginia pine. HEPTING, GEORGE H., AND GEORGE B. CUMMINS. A new species, *Peridermium (Cronartium) appalachianum*, is described on stems and branches of *Pinus virginiana*, in Virginia, West Virginia, North Carolina, and Tennessee. The aecia are large, columnar, and mostly nonconfluent, lack intrasoral filaments, and cause little or no hypertrophy. The uniformly verrucose aeciospores measure (11–) 13–19 (–22) × (19–) 29–43 (–53) μ. Spermogonia have not been observed. Inoculations on sweetfern and Virginia pine gave no infection. The oldest infections found occurred about 1936. A few trees have been found killed by the rust. The

rust forms cankers on the stems. No alternate host has been found.

KF 467 (ethyl mercuric perthiocyanate): A new chemical for seed treatment of vegetables and cereals. HEUBERGER, J. W. Research during 1942–1950, inclusive, sponsored by the Koppers Company, has resulted in the development of an effective, nonphytotoxic chemical (ethyl mercuric perthiocyanate; coded KF 467) for seed treatment. Tests showed: KF 467 (10 per cent active), 2 oz./bu., was equal to Spergon, 2 oz./bu., on peas (8 tests) and to Arasan, ¾ oz./bu., on sweet corn (4 tests); KF 467 (5 per cent active), 2 oz./bu., was equal to zinc oxide, 3 oz./bu., on spinach (5 tests) and to Spergon, 2 oz./bu., on lima beans. Tests on peas showed KF 467 to be nonphytotoxic even at 100 per cent active concentration. Storage studies with KF 467 (5 per cent active), 2 oz./bu., on peas, sweet corn, and spinach showed no phytotoxicity or diminution in effectiveness after 5 months. Cooperative tests showed that KF 467 (10 per cent active), 2 oz./bu., was: 1) equal to standard treatments on peas in 1949–1951, inclusive, in Wisconsin (D. J. Hagedorn); 2) tied for first rank, averaged data, on peas, spinach, beets, sweet corn, and lima beans in 1950–1951 inclusive, in Ohio (J. D. Wilson); 3) first in rank on drilled rice in 1949–1950, inclusive, in Louisiana (J. G. Atkins), but ineffective on water-planted rice; and 4) first in rank, averaged data, at either 1 oz., ¾ oz., or ½ oz./bu., of 40 treatments for wheat bunt, barley covered smut and false loose smut, oat smut, wheat seedling blight, and flax seed rot in the 1950 Cooperative Seed Treatment Trials in Canada and the United States (J. E. Machacek).

Effect of medium on sporulation and pathogenicity of pathogenically different single conidial isolates of Alternaria solani. HIGGINS, DANIEL J. Two single conidial isolates of *Alternaria solani* were made from each of four mass cultures. These single conidial isolates were then cultured on potato-dextrose agar, Difco potato-dextrose agar, and an agar medium containing juice of tomato plants. The isolates differed from one another in the number of conidia produced and in pathogenicity. Differences in conidial production occurred in the individual isolates with culture upon different media. Media appeared to have a definite antecedent effect upon the pathogenicity of certain isolates as measured by the number of lesions on young tomato plants.

Effect of tetrachloro-p-benzoquinone on certain fermentative enzyme systems in fungi. HOCHSTEIN, P. E., AND CARROLL E. COX. Manometric methods have been used to study the effect of tetrachloro-p-benzoquinone (Spergon) on certain phases of the metabolism of cells of *Fusarium solani* f. *pisi* (Jones) Snyder & Hansen and *Saccharomyces cerevisiae* Meyen. The effect of this fungicide upon two fermentative enzyme systems received primary consideration. When 5-mgm. aliquots of air-dried brewer's yeast cells were exposed in substrate solution to tetrachloro-p-benzoquinone at a concentration of 5.0×10^{-4} M, decarboxylation of pyruvic acid was completely inhibited. A similar effect upon the oxidation of alcohol to acetaldehyde was observed when cells of baker's yeast were treated in the same manner. The inhibiting effect of the fungicide upon both these enzyme systems was greatly decreased in the presence of either of the sulfhydryl-containing compounds, cysteine or glutathione. Since both carboxylase and alcohol dehydrogenase contain sulfhydryl groups, it is apparent that tetrachloro-p-benzoquinone acts to oxidize that group on these enzymes and is itself reduced. Other enzymes which contain sulfhydryl groups may be affected similarly. Gross responses of the *Fusarium* to the fungicide were similar to those of the yeast, and presumably similar enzyme systems are affected.

Early trials in oak wilt chemotherapy. HOFFMAN, PAUL. Several organic chemicals were applied to oak trees of various sizes, seedling to mature, of three species, *Quercus borealis* Michx., *Q. ellipsoidalis* E. J. Hill, and *Q. palustris* Muench., in 1949, 1950, and 1951 in an attempt to cure or prevent oak wilt. Bole injections, subsurface soil applications, and foliage sprays were tried. Root zone applications under pressure gave the best results. Selection of chemicals was based on spore germination inhibition and toxin-neutralizing capacity. Certain organic sulfurs, quaternary ammoniums, hydroxy quinolines, basic dyes, and organic mercury compounds were most effective against the oak wilt pathogen, *Chalara quercina* Henry. Field and greenhouse trials of 37 organic chemicals indicated that malachite green, calcium sulfamate, disodium ethylene bisdithiocarbamate, 8-hydroxy quinoline sulfate, sodium dimethyl dithiocarbamate + sodium 2-mercaptobenzothiazole, sodium dimethyl dithiocarbamate, 8-hydroxy quinoline benzoate, phenyl mercuri triethanol ammonium lactate, sodium 2-mercaptobenzothiazole, sodium trichlorophenate, alkyl dimethyl ethyl benzyl ammonium chloride, and cadmium succinate sufficiently retarded the disease to warrant further investigation. The effectiveness of several chemicals from a variety of organic groupings suggests a mechanism general in action rather than specific. The preponderance of basic chemicals in the foregoing list also suggests that this action may be one of neutralizing the toxin, since in neutral and alkaline pH ranges the toxin produced by the oak wilt fungus is inactive.

A rapid photometric method for determining generation time of fungi. HOLLIS, JOHN P. A photometer was constructed which enables measurement of light transmission through a Petri-plate culture. Spore suspensions of *Streptomyces scabies* (Thaxt.) Waks. & Henr. were spread over an agar surface, and light-transmission readings were taken during the exponential growth period. The increase in logarithm of dry weight was proportional to the photometer reading during the exponential growth phase. The change in photometer reading is proportional to the time over this period, and the slope of the curve (time vs. photometer reading) can be translated into generation time. The method is particularly applicable to a comparison of reactions of large numbers of strains of the same fungus species to different metabolites.

A paper-doll technique for disease tests of seed corn in cold soils. HOPPE, PAUL E. A simpler method than the glass-tumbler–paper-doll technique for extensive controlled germination and disease tests of seed corn in cold soils, without large refrigeration equipment or greenhouse facilities, has been devised. The kernels are placed on wet paper towels in contact with Pythium-infested soil spread thinly (1/16 in. deep) on the towels. The towels then are rolled into "paper dolls" and incubated, in covered metal pans, in an ordinary electric refrigerator for the desired time at 10°–11° C. Following the low-temperature incubation, the pans of seed are stored at room temperature and germinations are recorded 2 or 3 days later. This easy and rapid method of testing has proved efficient for the study of various aspects of the cold soil germination problem in corn.

The inhibition of wheat stem rust by sulfadiazine and the reversal of this effect by para-amino benzoic acid and folic acid. HOTSON, H. H. A 300-p.p.m. aqueous solution of sulfadiazine, plus 1 per cent Tween-20 as a wetting agent, sprayed on 7-day-old seedlings of Little Club and Spelmar wheats and Khapli emmer 3 days after inoculation with races 15 B and 56 of *Puccinia graminis tritici*, completely suppressed rust development and apparently did not injure the host. Six 4-in. pots containing 10 plants each could be covered with 20 ml. of the solution, producing incipient solution run-off from the plants. The rust-suppressing effect of sulfadiazine was reversed if a second spray of 300 p.p.m. of either para-amino benzoic acid or folic acid was applied within one day after the initial spray. With delay in applying the second spray, a gradual decrease in recovery occurred; with a delay until 5 days after the initial spray, no recovery of the rust was observed. Thus para-amino benzoic acid and folic acid are vitamins for wheat stem rust and probably para-amino benzoic acid is an intermediate in the biological synthesis of folic acid.

The use of tetraploids in breeding scab-resistant apples. HOUGH, L. F., AND J. R. SHAY. During 1949 and 1950,

pollen from three scab-resistant clones was used for controlled crosses with the giant-fruited tetraploid sport of McIntosh in the orchard of Roger Kimball, Littleton, Massachusetts. The 1364 seedlings obtained from these crosses were subjected to the routine artificial inoculations of this cooperative project. The ratios for resistant vs. susceptible in these progenies of presumably triploid seedlings are not significantly different from the ratios observed in progenies from the same resistant clones crossed with susceptible diploid varieties. It appears very likely that the dominant genes for resistance will be similarly effective in crosses with the tetraploid sports of other commercial varieties. These large-fruited sports of standard varieties are being used in backcrosses with advanced generation resistant selections which possess nearly acceptable size and quality. This procedure should produce scab-resistant seedlings of commercially acceptable size and quality in the next generation without the necessity of growing extremely large populations. It is recognized that it will not be practicable to use the triploid seedlings from such progenies for further backcrossing.

A mosaic disease of wheat, barley, and oats new to California. HOUSTON, BYRON R., AND JOHN W. OSWALD. In May 1951 a virus disease resembling Western wheat mosaic in some respects was found in several barley and wheat plants. The presence of the disease was not recognized soon enough to determine its distribution in 1951. The virus is readily transmitted to seedling wheat, barley, and oats when rubbed with expressed juice from diseased plants. Mosaic symptoms develop in 5 to 6 days at 70°–85° F. in the greenhouse. The mosaic pattern consists of yellow-green dashes and streaks usually limited by the larger leaf veins. Coalescence of numerous chlorotic dashes often produces large, irregularly shaped chlorotic areas in young leaves. Older plants tend to show more elongated interveinal streaks. Symptoms differing from other described wheat mosaics occur in the jointing stage. Kanred and Baart 38 wheats show extreme distortion with twisting and rolling of the upper leaves and failure of new leaves to emerge normally. Leaf, stem, and head distortion is extreme on barley C. I. 998, but not on other varieties tested. Ventura oats show the leaf distortion and curling at heading stage. Stunting is most pronounced in wheat, intermediate in oats, and slight in barley. There is no evidence of soil transmission.

A rag-doll method for mass testing of oat seeds for resistance to Helminthosporium victoriae. IVANOFF, S. S. A simple method for testing resistance to Helminthosporium blight has been developed which employs seeds in the laboratory instead of plants in the greenhouse or in the field. This method is particularly adapted to selection for disease resistance because of the facility it affords for screening out large masses of susceptible seeds before, or soon after, sprouting has developed. It consists in coating the seeds with an agar culture of the causal organism mixed with water in the proportion of 1 to 2, and in keeping the coated seeds in rag-dolls for 5–7 days at 100 per cent humidity and at 25° to 30° C. Susceptible seeds under these conditions either do not germinate or if they do germinate their radicles soon become necrotic and quickly disintegrate, while the resistant seeds germinate and develop as more or less normal seedlings which may be subsequently planted in soil and grown to maturity for seed. Hundreds of thousands of seeds may be tested with speed, uniformity, and high degree of accuracy. This laboratory method has been found equally effective with some other root-destroying pathogens.

Studies in the Myriangiales. III. Several little-known spot anthracnoses affecting trees. JENKINS, ANNA E., AND A. A. BITANCOURT. A spot anthracnose producing numerous small lesions on the upper surface of leaves of *Quercus falcata* has been found in Georgia. Similarly, what is evidently a corresponding disease is conspicuous on the upper surface of Ulmus sp. (said not to be an American elm) from Pennsylvania. The third related disease here reported affects *Inga edulis* in Central America. In this case the small leaf spots are visible on the lower as well as the upper surface of the blade. The pathogens of these diseases will be described as new. The fourth disease to be considered was discovered also in Central America and is possibly a spot anthracnose. This affects coffee berries.

Virus diseases of orchids in California. JENSEN, D. D., AND A. HERBERT GOLD. The flower breaking symptoms in Cattleya and its hybrids may be caused by one or more of at least two distinct viruses. One is transmissible mechanically to Cymbidium in which it produces mosaic leaf symptoms in approximately 6 weeks, but fails to infect *Odontoglossum grande*. Another virus produces ringspot symptoms in *O. grande* in 1 or 2 weeks and may be the same virus that was described under the name Odontoglossum ringspot. Odontoglossum ringspot virus can be transmitted by juice inoculation to Cymbidium in which it produces a symptom designated as diamond mottle. However, approximately 1 year elapses between inoculation and symptom appearance in Cymbidium in contrast to the short incubation period of 1 week in *Odontoglossum grande*. Odontoglossum ringspot virus also has been transmitted mechanically to and recovered from Zinnia in which it produces a transitory breaking, in the form of inconspicuous islets, in the immature flower petals. A virus of *Laelia anceps*, with symptoms consisting of brown to necrotic lines and partial or complete rings, is transmissible to Cymbidium in which it produces a leaf mosaic. Cymbidium is also susceptible to a virus originating in *Oncidium rogers*. In the latter this virus causes conspicuous, irregular chlorotic spots and streaks in the leaves.

Tobacco wildfire resistance in Wisconsin. JOHNSON, JAMES, AND ROBERT W. FULTON. Resistance to the wildfire disease (*Phytomonas tabaci*) has been developed in Havana Seed tobacco, which closely approaches Wisconsin commercial varieties. Based on number of plants diseased under the most favorable seed-bed and field conditions for infection, some of these new types are up to 98 per cent resistant. In other selections variation may occur in the degree of resistance as well as in the percentage of plants infected. The wildfire-resistant character was derived from Clayton's cross of *Nicotiana longiflora* and *N. tabacum*, namely T. L. 106. Crosses with Wisconsin types (Havana 307 and 322) resistant to black root-rot (*Thielaviopsis basicola*) were started in 1946. F$_2$ segregation for root-rot resistance (T. L. 106 susceptible), wildfire resistance, and agronomic features varied strikingly. Backcrossings have resulted in continued improvement in agronomic type while retaining the highest degrees of disease resistance. Good progress is also being made in combining mosaic resistance (NN type) with the wildfire- and root-rot-resistant types, thus assuring multiple disease resistance in one variety. These new strains are being tested under commercial conditions with promising results.

Recontamination of sterilized soil with relation to Pythium root rot of sugar cane and corn. JOHNSON, LEANDER F. Microorganisms isolated from sterilized, recontaminated soils were studied in relation to their antagonistic effect upon *Pythium arrhenomanes* Drechs., which causes a root rot of sugar cane and corn. Samples of autoclaved soils, some of which were artificially infested with *Pythium*, were exposed to the atmosphere and the populations of fungi, actinomycetes, and bacteria were determined by making dilutions of soil samples at various times. The organisms obtained were tested for antibiosis to *Pythium arrhenomanes* in culture. In order to determine the increase or decrease of pathogenicity of *P. arrhenomanes* in the recontaminated soils, corn was planted at the time the populations were determined. There was a decrease in Pythium root rot as the soils became recontaminated. The amount of root rot was generally reflected by decreased root weight and shoot height. No correlation between the Antibiotic Index (the average distance of inhibition in millimeters) of any of the three types of organisms and the amount of root rot was obtained; however, there was a definite correlation between the increasing Antibiotic Value (Anti-

biotic Index × number per gm. of soil) of the actinomycetes and the decreasing amount of Pythium root rot.

Asparagus rust investigations in Illinois. KAHN, ROBERT P., H. W. ANDERSON, AND PAUL R. HEPLER. The so-called rust-resistant Washington varieties of asparagus were demonstrated to be extremely susceptible to *Puccinia asparagi* in the greenhouse and field and may no longer be considered as commercially rust-resistant under Illinois conditions. No highly resistant strains were located in a collection of these varieties from 12 sources in the United States, in nine other named varieties including American and European representatives, and in 12 selections from Canada and South Carolina. However, a difference in susceptibility among these varieties and selections was observed. Fungicides including sulfur, ferbam, zineb, copper-zinc-chromate, phenyl mercury triethanol lactate, tribasic copper sulfate, or combinations of some of these fungicides were not effective in reducing the severity of rust epiphytotics in 1949-1951 when applied as dusts or sprays with ground equipment or as dusts from airplanes. In the absence of effective fungicides or resistant varieties, field observations indicate that coordinated planting and harvesting programs by all the growers in any one asparagus-growing center may eliminate primary infection or postpone secondary infection until near the end of the growing season.

Field investigations of sugar-beet seedling disease control. KAZMAIER, HAROLD E. The effects of rate and method of seed treatment and placement of fertilizer-fungicide mixtures were investigated in Ohio in 1950. Comparisons were made between refinery seed treatment (4½ lb. of Arasan dusted on 800 lb. dry seed) and a series of moistened seed lots (800, 600, 400, 200, and 100 lb. of seed) dusted at the rate of 4½ lb. of Arasan to each lot. Plantings involving fertilizer-fungicide placement were made with the Milton Beet Planter, placing the mixture either in direct contact with the seed or distributing it from top to base of the seed furrow by altering the position of the fertilizer delivery tube. Arasan was mixed with the fertilizer at the rates of 3 and 4 lb. per acre. Stand density was rated 0–36 depending upon the number of inches containing seedlings in 36-in. row intervals. The most effective rate of seed treatment was 4½ lb. of Arasan dusted on 600 lb. of moistened seed. Selection of the best method of placement of the fertilizer-fungicide mixture could not be determined from the results.

A "deep-freeze" method of maintaining virulent inoculum of the alfalfa wilt bacterium, Corynebacterium insidiosum. KERNKAMP, M. F., AND GLEN HEMERICK. Maintenance of virulent inoculum of *Corynebacterium insidiosum* is difficult because the organism frequently loses its virulence after several months in culture. A "deep-freeze" method of maintaining virulent inoculum in host tissue was developed. Tap roots infected with the organism were stored in a home freezer at –20° C. for 21 months, and virulent cultures were readily isolated from them. Inoculum of even greater virulence was obtained when roots were ground in a food chopper and the material was soaked in water for 12 hr. In greenhouse tests, 4-month-old seedlings, grown under continuous light, were inoculated with bacterial suspensions from macerated frozen roots, from cultures isolated from frozen roots, and from cultures that had been on agar approximately 6 months. The bacterial suspension from macerated frozen roots caused moderate to severe infection in 6 weeks; bacteria that were isolated from frozen roots and bacteria from agar cultures 6 months old caused only weak to moderate infection, and the incubation period was prolonged to 8 weeks. The deep-freeze method offers a simple means of maintaining a source of virulent inoculum without the necessity of isolating the organism and growing it in culture.

Oak wilt development in relation to time and place of inoculation and concentration of inoculum. KUNTZ, J. E., C. H. BECKMAN, AND A. J. RIKER. Factors influencing the development of oak wilt symptoms were studied. Root-inoculated northern pin oaks (65,000,000 spores per tree) developed widespread incipient wilt in 3 weeks, regardless of the date of inoculation (May 23 through September 6). *Chalara quercina* Henry was often isolated from the entire tree a week before and consistently at the time of initial symptoms. Moreover, the reductions of upward movement of radioactive rubidium ($Rb_2^{86}CO_3$) and of water flow through twig sections were confirmed. In 5 weeks, severe wilt developed. Oaks inoculated in a low, lateral branch likewise developed incipient wilt in 3 weeks. However, initial symptoms and often *Chalara* were restricted to inoculated branches. Neither rubidium nor water moved through these wilting branches although such movement was normal elsewhere. With these lateral-branch inoculations, wilt symptoms progressed unilaterally upward into the terminal. The entire crown wilted only after 7 weeks. Terminally inoculated oaks also showed incipient wilt in 3 weeks but severe wilt was delayed until 7 weeks. Incubation periods increased as spore loads decreased. At low spore concentrations (6500 to 65 spores per tree), branch-inoculated trees showed symptoms sooner than root-inoculated trees. Differential responses to places of inoculation and spore loads have implications for long-distance spread.

Studies on the fungicidal activity of helixin. LEBEN, CURT, AND G. W. KEITT. The protectant action of a partly purified preparation of helixin, an antibiotic previously shown to inhibit fungi and certain bacteria, was studied in five greenhouse tests with *Alternaria solani* on tomato foliage. Sprays were adjusted to pH 5.7–6.0. The average LD50 and LD 95 were, respectively, 6.5 and 37.9 μg. per ml. The disease control at 45 μg. per ml. with sprays buffered at pH 3.0 and pH 6.0 was 70.7 and 96.3 per cent, respectively. The pH also influenced activity in agar-streak tests: with each of seven test organisms, the antibiotic was more inhibitory at pH 7.4 than at 4.7. By means of a leaf-disk assay similar to that described for antimycin, it was shown that the deposit from a spray at pH 6.0 was completely washed from the leaf by 12 mm. of simulated rain; when a deposit on leaves was sprayed with a weak acid solution, however, 26.3 per cent of the activity remained after washing. On the acid-sprayed plants 42 per cent of the original activity remained after 4 days, as determined by leaf-disk assays.
mined by leaf-disk assays.

Paratylenchus hamatus Thorne & Allen associated with celery disease in Connecticut. LOWNSBERY, B. F., AND J. W. Lownsbery. Fields of stunted and chlorotic celery were found to be infested with 1000 to 8000 *Paratylenchus hamatus* per 250 cc. of soil. Larvae and adults of *P. hamatus* were observed feeding on all parts of the celery root system, and on dill. Eggs of *P. hamatus* were found on the root surface around feeding females. Larvae and adults survived the 1950-1951 winter in the field. This nematode has been previously reported only on fig in California. In pot tests celery plants grown in this soil treated with methyl bromide weighed four times as much as plants grown in the nontreated soil. Addition of 2500 *P. hamatus* to potted celery plants growing in soil previously treated with methyl bromide resulted in significant reduction in weights of these plants as compared to non-infested methyl bromide-treated checks. Infested roots showed stunting and necrosis proportional to the nematode population. A *Rhizoctonia* was isolated from many of the lesions and may contribute to the disease.

Longevity of some common fungi in cereal seed. MACHACEK, J. E., AND H. A. H. WALLACE. Tests made at regular intervals over a period of 10 years on naturally infected seed of wheat, oats, and barley that had been held in storage showed that most of the seed, while retaining its germinability, became free from viable fungi before the end of the storage period. *Alternaria tenuis sensu* Wiltshire in all three crops, *Helminthosporium sativum* P. K. & B. in wheat and barley, and *Septoria nodorum* Berk. in wheat died out comparatively rapidly. *H. avenae*

Eidam in oats and *H. teres* Sacc. in barley lost their viability slowly and since, by the end of the storage period, most of the microorganisms associated with them in infected kernels were dead, these two fungi could generally be recovered in pure culture.

New evidence for multiplication of aster-yellows virus in its vector. MARAMOROSCH, KARL. In a serial-passage experiment of aster-yellows virus in the vector *Macrosteles divisus* Uhler, measurements of virus concentration were made in samples of insect juices used for the injection of the 1st and 9th group of leafhoppers. The measurements employed a new method, based on the increase of incubation periods in insects injected with decreasing amounts of virus and tested under controlled environmental conditions. Individual inoculated leafhoppers, fed at 25° C. through screens of small cages fastened to the leaves, were transferred to new sets of China asters at intervals of 1 to 3 days. Noninjected insects from the same stock served as controls and proved virus-free. Exposed plants were observed in a greenhouse for 6 weeks. The lengths of incubation periods in the 1st (24 to 38 days) and 9th (23 to 29 days) group were similar. Therefore, it was concluded that concentration of virus was not decreased after eight passages, during which the insects had no access to fresh virus. Thus new and direct evidence for multiplication of this plant virus in its insect vector confirmed earlier findings.

Fall spraying in the control of bacterial spot of peaches. MASON, CURTIS L. Preliminary tests in the fall of 1949 indicated beneficial results in the control of bacterial spot of peaches (incited by *Xanthomonas pruni*) by spraying with copper sulfate and water. In the fall of 1950 several experimental plots were sprayed in this manner. The control of bacterial spot was very good in all tests but some copper injury was encountered in a few instances. Later it was determined that in each case of injury, the trees had been sprayed soon after a rain when the relative humidity was very high. In other cases, no injury was encountered. In the spring of 1951 no disease was found on the sprayed plots until 2 weeks after it was first found on the checks. During this time, there were several hard blowing rains. As a result, some infection occurred over the entire sprayed area except where the sprayed areas were upwind from the unsprayed areas. In the latter very little disease developed, while adjacent downwind checks were severely defoliated. In all sprayed plots there was less disease on both foliage and fruit than on comparable unsprayed checks.

Fungitoxicity of compounds possibly concerned in the mode of action of sulfur and ferbam. MCCALLAN, S. E. A., LAWRENCE P. MILLER, AND RICHARD M. WEED. The compounds involved are difficult to assess properly because of easy interconvertibility, e.g., between sulfur and hydrogen sulfide, low solubility and high volatility, e.g. carbon disulfide. Hence, their fungitoxicity has been reexamined by technics designed to overcome some of these handicaps. Studies were made with spores of *Sclerotinia fructicola*, *Glomerella cingulata*, *Aspergillus niger*, *Neurospora sitophila*, *Cephalosporium acremonium*, *Alternaria oleracea*, *Rhizopus nigricans*, and *Stemphylium sarcinaeforme*. Physical form of sulfur is most important; colloidal sulfur had ED50 values from 0.1 to 150 p.p.m. depending on the species, whereas wettable sulfur was nontoxic to all except *Sclerotinia*. For most species hydrogen sulfide was less toxic than colloidal sulfur. Ferbam was more toxic than ethylene diamine or dimethylamine; carbon disulfide was relatively nontoxic. Eight strains of *Neurospora crassa* exhibited a similar order of sensitivity to wettable sulfur and ferbam. However, the dosage response curves are much steeper for ferbam. Concentrations of sodium arsenite, found in other tests to completely stop the production of hydrogen sulfide by spores from sulfur, when added to sulfur did not decrease its toxicity. These results indicate that the role of hydrogen sulfide in sulfur toxicity is less than previously supposed. Atomic Energy Commission support is acknowledged.

Experiences with cherry sprays in 1951. MCCLURE, THOMAS T. Sprays to control leaf spot (*Higginsia hiemalis*) were applied to different blocks of sour cherries at East Lansing and Shelby, Michigan. Ferbam, Orthocide 406, Glyoxalidine 341, Actidione, manganese bis-dithiocarbamate, nabam-zinc, and tribasic copper sulfate-lime were compared. At East Lansing, three preharvest sprays applied thoroughly and on time gave excellent control until October 1 regardless of materials except for trees adjacent to unsprayed controls. On trees exposed to nearby inoculum, infection and defoliation became noticeable beginning September 1. At Shelby in a similar program, Orthocide 406 and Actidione 2 p.p.m. gave the best control, with Glyoxalidine next best. Ferbam-sprayed trees had considerable infection and defoliation after September 1 with heavy defoliation on individual trees by October 1. Actidione in three sprays beginning at petal fall stopped growth and development of fruits, giving many small immature cherries at harvest time. How early this material can be safely applied is a question. On sweet cherries Actidione following ferbam caused marked injury. Manganese bis-dithiocarbamate caused some leaf yellowing early in the season.

A species of Aphanomyces causing damping-off in peppers and certain other vegetables. MCKEEN, C. D. Several cultures of a species of *Aphanomyces* were isolated from damped-off pepper seedlings obtained in two commercial greenhouses in 1950 and again in 1951. With respect to vegetative characters and morphology of the sexual organs this fungus corresponded closely with the description of *Aphanomyces cladogamus* given by Drechsler. The early infection of pepper seedlings by *Aphanomyces* might be readily mistaken for attack by *Pythium* sp. or even *Rhizoctonia solani*. However, later post-emergence attack by *Aphanomyces* is distinct in that the hypocotyl turns black and often collapses near the point of origin of the cotyledons. The blackened area frequently extends into the base of the cotyledons and often down one side of the hypocotyl. Whereas only a few mycelial strands of this fungus were observed infrequently in the radicle and root tissues, the hypocotyl and cotyledonary tissues were colonized extensively. *A. cladogamus* was capable of causing considerable damping-off in tomatoes, radish, and eggplant, but was not observed to attack lettuce, onion, or muskmelon seedlings. Damping-off in peppers, as caused by this fungus, was much more effectively controlled by treating the seedbed soil, rather than the seed, with thiram.

An aberrant strain of the black rot fungus causing fruit and stem rot of greenhouse cucumbers in Ontario. MCKEEN, C. D. In 1950 an unusually severe outbreak of stem rot of cucumbers occurred in a single large greenhouse. The type of parasitism displayed as well as the morphology of the causal organism indicated it to be closely related to the black rot fungus (*Mycosphaerella melonis* (Pass.) Chiu & Walker). Greenhouse experiments demonstrated that the fungus always became established either on senescent or moribund plant parts, and in the presence of high humidity readily invaded and destroyed healthy tissue. Although pycnidia were produced in abundance on most lesions, perithecia were found rarely, and never interspersed among pycnidia. While this fungus agreed closely with descriptions of the black rot fungus given by several previous investigators, still greater variability and pleomorphism than recognized hitherto was demonstrated by the existence of two different characters. First, perithecia were found to bear near the ostiole a ring of setae of variable length and number. In old cultures the setae were somewhat inconspicuous. Second, a few pycnidia that developed on naturally infected stems as well as in artificial culture produced two types of conidia, viz., Alpha and Beta conidia. The ratio of the two spore types varied greatly from one pycnidium to another.

Interaction between fungus spores and sulfur, ferbam,

and 2-heptadecyl-2-imidazoline as shown by radioactive tracers. MILLER, LAWRENCE P., S. E. A. MCCALLAN, AND RICHARD M. WEED. Fungus spores and mycelium showed great affinity for sulfur as indicated by the large amounts taken up when they were exposed to sulfur vapor. Radioautographs showed that when lilac leaves infected with *Microsphaera alni* are exposed to the vapor of sulfur, much more is taken up by the mycelium than by host tissue. Absorption of sulfur by spores resulted in almost immediate release of hydrogen sulfide. Rate of hydrogen sulfide formation was affected 100-fold by the physical form of the sulfur used, and the rate varied somewhat over 100-fold among spores of nine species. With some species practically all of the sulfur applied can be recovered as hydrogen sulfide. The quantity of sulfur required per spore to prevent germination was determined for some species. Dosage of fungicide received by spores is determined by the relation between the number of spores present and the total amount of chemical available, and the dosage received may be markedly higher than indicated by the concentration applied. 2-Heptadecyl-2-imidazoline (glyoxalidine) was especially rapidly removed from solution by spores, and effective dosages of about 2000 mgm. per kilogram were reached with solutions containing only 2 mgm. per kgm. Atomic Energy Commission support is acknowledged.

Control of cracking in sweet potato by soil fumigation. MULLIN, R. S. Soil fumigation tests were made in 1950 in an attempt to control cracking in sweet potatoes in eastern Virginia. The material used, dichloropropene-dichloropropane, was put in the soil with a hand injector in five 15-ft. by 50-ft. replications. Sweet potatoes produced in the same land in 1949 had been infected with nematodes, both in the rootlets and in the potatoes, and cracking and black eyes had been severe. Results of the 1950 experiment with Porto Rico variety were 14.69 per cent of the potatoes cracked in treated areas, and 51.18 per cent cracked in nontreated. Nematodes were present in the rootlets and potatoes in both areas, but to a much greater extent in those from the nontreated areas. It is evident that in this field, nematodes have been a factor in cracking of sweet potatoes.

Control of onion maggot and smut by one treatment at seeding time. NEWHALL, A. G., W. A. RAWLINS, AND M. J. SLOAN. The addition of an insecticide to the 4 per cent methyl cellulose solution employed in coating onion seed with the fungicide Tersan for onion smut control has resulted in successful control of the onion maggot, *Hylemia antiqua,* in several replicated field tests on the mucklands of New York State. Chlordane and Heptachlor were the most effective tried, the quantities required being less than 100 gm. technical to the pound of seed per ⅛ or ¼ acre. Dieldrin and lindane were somewhat less effective. Systox, a systemic insecticide, gave poor control, and insectary tests indicate DDT and toxaphene to be ineffectual. Onion smut was very prevalent in 1951. In comparison with liquid formaldehyde, Vancide 51 gave good control in approximately 1 per cent solutions. Several dry fungicide-insecticide combinations blown into the furrow with the seed also gave excellent smut and maggot control in a number of field tests. The better ones included Orthocide-Dieldrin, thiram-Heptachlor, Tersan-Chlordane, and Tersan-Parathion. Trials indicated that 20 lb. of a dust containing 3 lb. of fungicide (thiram) to 3 lb. of the insecticide (Heptachlor 50 per cent active) in 14 of Pyrax is sufficient for an acre, or 6 mi. of row.

Control of onion blast and mildew. NEWHALL, A. G., RALPH SEGALL, W. A. RAWLINS, AND M. J. SLOAN. From 3 to 12 applications of liquid nabam-type carbamate (Dithane D-14) or its zineb dust counterpart (Dithane Z-78) plus an insecticide for thrips control were made at approximately weekly intervals with power sprayers and dusters on 14 muckland onion farms in six New York counties, beginning late in June. Treated onions remained green 2–4 weeks later and yielded an average of 162 bags (of 50 lb.), or 26 per cent more than untreated, lowest increase being 38 bags or 10 per cent, and highest 277 or 43 per cent. Most of the increase was due to blast control, as thrips never became very troublesome. This and other information indicates blast may be caused by fungi hitherto considered unimportant. On three fields in well-replicated spray vs. dust plots, spraying 5, 6, and 12 times gave increases of 277, 243, and 246 bags (average 42 per cent); dusting gave 196, 189, and 183 bags increase (average 30 per cent) respectively. The addition of 30 per cent dusting sulfur to the 7 per cent Z-78 dust failed to enhance control of blast. Timing of applications in three cases seemed very important. On one farm nine applications of liquid Parzate gave good blast and mildew control; manganese ethylene bis dithiocarbamate spray was less effective. Mildew appeared on three farms but was well controlled by both spray and dust. In two tests on early set onions no disease occurred and no benefits from spraying or dusting resulted.

Reduction of the incidence of black shank in resistant tobacco varieties by soil fumigation. NUSBAUM, C. J., AND J. F. CHAPLIN. Black shank sometimes causes unusually high losses in plantings of resistant, flue-cured varieties of tobacco. Root invasion by parasitic nematodes often is suspected as a contributing factor. In a field naturally infested with *Phytophthora parasitica* var. *nicotianae,* black shank development was studied on five tobacco varieties on fumigated and nonfumigated soils. The varieties were 402 and 116-99, both susceptible to black shank; Bottom Special, tolerant; Dixie Bright 101, moderately resistant; and Dixie Bright 102, highly resistant. All except 116-99 are susceptible to root-knot nematodes. On treated plots, ethylene dibromide (Dowfume W-40) was applied at 20 gal. per acre. Fumigation for nematode control had little effect on black shank development in susceptible varieties, all plants being killed by July 28. The incidence of black shank in Bottom Special was reduced by treatment early in the season, but none of the plants survived. In both resistant varieties, especially Dixie Bright 101, black shank developed later and was less severe in fumigated than in nonfumigated plots. While the mode of action of the fumigant in reducing the incidence of black shank in resistant varieties was not determined, a nematocidal rate of application was effective.

Barley yellow-dwarf, a virus disease of barley, wheat, and oats readily transmitted by four species of aphids. OSWALD, JOHN W., AND BYRON R. HOUSTON. A disease of barley characterized by brilliant leaf yellowing and dwarfing of the plants occurred in epiphytotic proportion in the 1951 California crop. Investigations have shown that this disease, named yellow-dwarf, is caused by a virus which is readily transmissible by four species of grain-infesting aphids, *Macrosiphum granarium, Rhopalosiphum (Aphis) maidis, Rhopalosiphum prunifoliae,* and *Macrosiphum dirhodum.* Ten viruliferous aphids were caged on each seedling and allowed to feed for 3 days with the following results: the grain aphid, *M. granarium,* 150 plants inoculated, 129 infected; the corn aphid, *R. maidis,* 114 inoculated, 71 infected; the apple-grain aphid, *R. prunifoliae,* 214 inoculated, 192 infected; the grass aphid, *M. dirhodum,* 71 inoculated, 19 infected. In no instance did nonviruliferous aphids of any of the four species cause any disease to develop. Research to date indicates the virus is not mechanically transmissible. The barley yellow-dwarf virus also affected the commercial crop of wheat and oats in 1951. In wheat the virus causes severe stunting and an abnormally light green color of the foliage. A severe reddening of leaves accompanied by extreme stunting is the principal effect of the yellow-dwarf virus on oats.

Effect of potassium nutrition and amount of inoculum on rate of reproduction of the root-knot nematode Meloidogyne incognita. OTEIFA, B. A. Lima-bean plants grown in sand culture were inoculated with the root-knot nematode *Meloidogyne incognita.* Three nutrient treatments were employed supplying low, intermediate, and high potassium

concentrations. Two levels of nematode inoculum were used at rates of 50 and 200 egg masses. Results of the experiment showed that differences in the number of female nematodes produced on the roots and difference in the rate of their oviposition can be induced by variation in potassium concentration. Among plants receiving a relatively low amount of inoculum, rates of nematode reproduction were apparently limited by the amount of potassium available. In plants receiving relatively higher inoculum and treated with higher potassium concentration, rates of reproduction are correlated with the amount of root available and with competition between nematodes for root space rather than with the amount of potassium available.

Needle droop of red pine. PATTON, R. F. An abnormal drooping and dying, called needle droop, of current season's needles of trees in young red pine plantations in Wisconsin has been attributed particularly to drought, heat, frost, or insects, or to infection by *Pullularia*. Field and greenhouse inoculations made with *Pullularia* under various conditions on over 40,000 needle fascicles never have induced symptoms of needle droop. These results indicated that the fungus played little, if any, part in the initial cause. To examine the physiological aspect, experiments were conducted with 4-year-old potted trees on the effects of drought, water-soaking, beating rain, high temperature, physiological drought, and rapid transpiration in association with low soil-moisture content. Typical symptoms appeared in trials favoring rapid drying of the needles and stems. They were reproduced commonly by rapid transpiration with a limited moisture supply. These experiments pointed to an explanation of needle droop in the field. With limited soil moisture, excessively rapid transpiration led to an absorption lag. The result was a serious internal water deficit and loss of turgor. Succulent tissue near the needle base collapsed, the needles drooped and died, and in extreme cases the twigs died. *Pullularia* appeared promptly as a secondary invader of the dead tissue.

A bark canker disease of London plane, redbud, and sweet gum. PIRONE, P. P. A highly virulent bark disease of London planes, *Platanus acerifolia*, growing along New York City streets has been under investigation since 1947. Spore dilutions from pycnidia of a Dothiorella stage on infected bark, as well as plantings of discolored inner bark and outer sapwood, have consistently yielded the same fungus. The perfect stage has never developed on artificial media. Wound inoculations showed that this fungus is capable of infecting sweet gum, *Liquidambar styraciflua*, and redbud, *Cercis canadensis*, in addition to the London plane, the species from which it was isolated originally. A culture of *Botryosphaeria ribis* from *Cercis canadensis* supplied by Ross W. Davidson was also found to be pathogenic to the three above-mentioned tree species. Although both fungi are capable of infecting the same three hosts, they appear to be different when grown on potato-dextrose agar. Studies are being continued to determine whether the two fungi are distinct species or whether they are strains of the highly variable *Botryosphaeria ribis*.

Control of corky excrescence of camellia. PLAKIDAS, A. G. Since previous studies indicated corky excrescence to be a nonparasitic disease caused by fluctuations in soil moisture, certain sprays designed to reduce transpiration were tried. A wax emulsion (Dowax 222) was tried in 1949. Three applications, June 6, July 6, and August 27, were made with 1 part in 25 parts of water. Complete control was obtained. In 1950, the same material was used in 1:30 dilution in two separate localities. Four applications, June 14, July 17, August 11, and September 14, were made in one locality; the July spray was omitted in the second locality. Practically 100 per cent control was obtained. Only a very slight degree of leaf burning occurred in both years. In 1951, the emulsion was used in 1:50 dilution. Three applications (June 26, August 6, and September 8) were made on one lot of plants, two (August 6, September 8) on a second, and one (September 8) on a third. Complete control was obtained in the first two lots but only partial control in the third. Severe leaf burning occurred from the first two sprayings but only a trace of injury from the third. In another test, "Good-rite Latex VL 600," 1 to 10 in water, was used in three applications with nearly complete control and no apparent injury.

Reaction of oat varieties to black stem and to a toxic substance produced by the causal organism. POOLE, D. D., AND H. C. MURPHY. The common name "black stem" is proposed for a disease originally named "speckled blotch" of oats by G. F. Weber in 1922 and caused by *Leptosphaeria avenaria* Weber. Since stem infection appears to be the most important phase of the disease and the symptoms produced at this stage are not easily confused with other diseases, the name black stem is preferred. This black stem phase is characterized by grayish brown to shiny black lesions on the upper internodes, particularly underneath the leaf sheath. Many varieties exhibited severe culm breaking at these lesions as maturity approached. The varieties Andrew, Marion, Kent, Cherokee, Nemaha, Mindo, Benton, Colo, Zephyr, and Bonham were the most susceptible, while Richland, Mo. 0-200, Clinton, Shelby, Bonda, Mo. 0-205, Branch, Ajax, and Beaver were moderately resistant to resistant, to the black stem phase, in uniform oat nurseries grown throughout Iowa in 1951. Since most of these resistant and susceptible varieties are of similar parentage, resistance is probably conditioned by multiple gene action. Cultures of this organism show a characteristic browning of the substrate when grown on a number of media containing dextrose. When sprayed onto the leaves or injected into internodes of oat plants, an extract of this substrate produces typical leaf blotch and black stem symptoms.

Studies on resistance to cucumber virus 1 in spinach. POUND, GLENN S., AND PEN-CHING CHEO. In crosses between Virginia Savoy and Nobel spinach varieties, resistant and susceptible, respectively, to cucumber virus 1, resistance was completely dominant in the F_1. Segregation in the F_2 approximated a 3:1 ratio, indicating a single dominant gene. Resistance was expressed only at air temperatures below 28° C. At 28° and above, plants rapidly succumbed to a systemic necrosis which began in the apical meristem. At 16° and 20° inoculated plants showed no symptoms and the virus was recovered from them within 2 weeks after inoculation but not after long intervals of time. At 24° inoculated leaves showed symptoms but no systemic symptoms occurred. Soil temperature, photoperiod, light intensity, and host nutrition did not alter this susceptibility at high air temperature, although slight increases or decreases in the amount occurred. A 16-hr. exposure per day at 28° was necessary for the production of top necrosis.

Complement fixation as a method of differentiating mutant strains of tobacco mosaic virus. PRICE, W. C., AND ERNEST P. WEAVER. Tests carried out with antisera produced in rabbits with six strains of tobacco mosaic virus show that the complement fixation reaction can be used in three different ways to differentiate mutant strains of this virus. First, antibody remaining after absorption of specific antiserum with heterologous antigen can be detected by complement fixation in the presence of homologous antigen. Second, strains sufficiently unlike show a lower antiserum dilution end-point in heterologous reactions than in homologous reactions. Finally, strains appear to differ in weights of antigen required to react optimally with specific immune serum. The latter permits differentiation with purified virus preparations only.

Leaf penetration by Phytophthora infestans. PRISTOU, R., AND M. E. GALLEGLY. The mode of penetration by the common potato race of *Phytophthora infestans* was studied on leaves of tomato (Wisconsin 55) and resistant and susceptible varieties of potato (Kennebec and Cobbler). Leaflets inoculated with drops of zoospore or sporangial suspension were incubated at 20° C. for 2, 6, 12, 24, 48, and 96 hr. At each period some leaflets were sectioned freehand; others were fixed in formalin-acetic acid. Prior to fixation the inoculated leaves were sprayed with egg albumen to prevent zoospore wash-off. All paraffin sections were stained with a modified version of Conant's quadruple stain. Observations showed that zoospores always produced an

appressorium before penetration. The appressorium, closely affixed to the epidermal cell, was approximately the same size as the zoospore and was separated from the germ tube by a cross wall. After appressorium formation the zoospore and germ tube were void of protoplasm. An infection peg entered through a small opening in the epidermal cell. The entire process of zoospore germination, appressorium formation, and penetration often occurred 2 hr. after inoculation. All stages of germination and penetration were observed at the 6-hr. period. Penetration in this manner occurred on leaves of resistant and susceptible potato varieties and the tomato.

Effect of certain environal factors on the initiation and development of the white rust disease of spinach. RAABE, ROBERT D. In studies on the white rust disease (*Albugo occidentalis*) of spinach, it was found in regard to air temperatures that the rate of disease development paralleled a temperature gradient, being most rapid at 28° C. and least rapid at 16° C. However, the lower temperatures were more favorable to conidial production and spread of the pathogen. In the greenhouse, oospore production was abundant and was favored by the warmer temperatures; more oospores were produced in seed plants than in vegetative plants. In laboratory germination studies, conidia germinated best in double-distilled water at a temperature near 12° C. Germination occurred, however, from 2° to 25° C. Chilling the conidia for 1½ hr. at 12° C. increased germination and resulted in about equal germination at all temperatures. Only conidia from opened pustules germinated well, indicating that germination depended somewhat upon a certain degree of maturation. Germination occurred at pH levels from 3.0 to 10.7 with an optimum near neutrality. Conidia taken from wilted plants or from detached leaves germinated better than those from turgid plants or leaves left intact on plants. In such instances, germination increased with an increase in water loss up to a certain point, beyond which little or no germination occurred.

Bayberry yellows. RAYCHAUDHURI, S. P. A presumably new virus causing symptoms of yellows disease in bayberry (*Myrica carolinensis* Mill.) was observed in New Jersey near Pemberton in two locations. The disease is characterized by yellowing, stunting, and often by a witch's-broom appearance. Sterility is normally associated with the malformation. The virus was transmitted by grafting but not by juice inoculations. By the agency of dodder (*Cuscuta campestris* Yuncker) the virus was taken to periwinkle and carrot, in which it produced yellows symptoms. Attempts to transmit it to China aster or to alfalfa by this method were unsuccessful. Flowers of infected periwinkle plants were variously malformed and virescent. Other symptoms in periwinkle were not identical with symptoms of any of the known yellows diseases studied earlier in this host. *Macrosteles divisus* Uhler failed to transmit the virus to aster and carrot plants.

A simple method of testing barley in the field for susceptibility to scald. REED, H. E. Scald caused by *Rhynchosporium secalis* causes considerable damage to barley in Tennessee. Moderately spotted leaves are usually killed when temperatures fall to freezing or below, and this has often been erroneously attributed to winter injury. Natural occurrence of the disease under local conditions is uncertain, hence a method was devised to increase the probability of an epidemic in barley plots. Test plots were seeded in August with a highly susceptible variety in every eleventh row. In mid-September barley straw from plants severely diseased with scald the preceding spring and held in dry, cool storage was scattered on the test plots. The disease occurred early with a consequent build-up and by April was uniformly severe throughout. By this method 150 different lines, replicated three times, were evaluated in 1950–1951 and a few showed marked resistance. Near-by plots to which no diseased plant straw was applied had no disease.

Lipid-solubility and fungitoxicity. RICH, SAUL, AND JAMES G. HORSFALL. The Overton-Meyer theory, now generally accepted, states that penetration through semipermeable membranes depends on partial solubility of the compound in both the lipid and water phases of the membrane. In order for a fungitoxicant to penetrate to its site of action, it must, therefore, have the proper balance of lipid-soluble and water-soluble moieties if the above theory applies. The fungitoxicity of derivatives of 2-imidazoline is enhanced by the addition of hydrocarbon substituents, as shown by Wellman and McCallan. Studies in our laboratory indicate that this effect is caused by increasing ability to penetrate, rather than a change in toxic mechanism, since the dosage-response slopes of the homologues were essentially parallel. 2-Dodecyl-2-imidazoline gave maximum phytotoxicity, while 2-heptadecyl-2-imidazoline gave maximum fungitoxicity in the studies of Wellman and McCallan. It is conceivable that this phenomenon is caused by differences in permeability of the semipermeable membranes of host and parasite. In studies with homologues, increasing fungitoxicity by the addition of carbon atoms to the side chain usually reaches a maximum, after which the addition of more carbon atoms results in decreasing fungitoxicity. These peaks in fungitoxicity suggest that the most active compounds have the optimum ratios of lipid-solubility to water-solubility for maximum penetration.

Intermediate reaction to race 45 of crown rust, and rust-escaping qualities exemplified in the new Bond derivative, Arkwin. ROSEN, H. R. In addition to the usual reactions to race 45 in oats with Bond parentage, there appear to be at least two other types of reaction in some Bond derivatives when subjected to artificial or natural epidemics of race 45 in the field. One of these consists of a reaction in which the pustules are usually smaller than those found in a 3 or 4 infection type, with a marked reduction in the number of spores per pustule. This is designated here as an intermediate type reaction and is comparable to that found in some Victoria derivatives reacting to this race. The other consists of a marked reduction in number of infections, comparable to the well known reactions of Red Rustproof strains of oats to race 1 in the field (but not in the greenhouse). This is designated as rust-escaping (klendusity) and is seemingly possessed by oats of diverse parentage including, in addition to the Red Rustproofs, Mutica Ukraina (Ukraine), Rainbow, and Ajax; and to a certain extent by such Bond derivatives as Shelby and Cherokee. It is also possessed by the new winter oat, Arkwin, as revealed in the presence of a severe epidemic of crown rust, mostly race 45, in the fall of 1950.

Factors and results in the partial-vacuum inoculation of seedling corn with Ustilago zeae. ROWELL, J. B., AND J. E. DEVAY. A partial-vacuum method of inoculating corn seedlings with smut has been tested as a means of quantitatively determining the characteristics of pathogenicity in *Ustilago zeae*. Uniform results required inocula of 10 million cells per ml. prepared from unicellular, logarithmic phase shake cultures grown on complete media, and aseptic seedlings with coleoptiles 1 cm. long which were wounded by a cut at the tip of the leaf sheath. Rate of disease development in planted, inoculated seedlings depended on temperature, galls forming in 10, 14, and 19 days at approximate temperatures of 80°, 70°, and 60° F., respectively. No differences in disease response to the cross of the haploid lines 17D4 × 10A4 were found in tests of 20 corn lines, crosses, and varieties representing extremes of field resistance and susceptibility. Marked differences in pathogenicity were demonstrated between the haploid cross 17D4 × 10A4 and the two solopathogens 410qq and 410N. The former produced severe stem galls which developed in 92 per cent of the plants within 10 days. The solopathogens produced mostly leaf galls which required 21 days for complete development; and only 69 and 49 per cent of the plants were infected respectively by 410qq and 410N.

Identification of root-knot nematodes (Meloidogyne spp.) by host tests. SASSER, J. N. Host range studies were conducted with the five described *Meloidogyne* species which occur in the United States. Approximately 25 agronomic crops were tested. Inoculum for each species was the progeny of a single female. Tomato seedlings, highly susceptible to all five species, were used as controls. The

tomato plants received the same amount of inoculum as the test plants. The susceptibility of the test plants was determined by comparing the amount of galling on their roots with that on the tomato controls. The tests revealed that some plant species are highly resistant or immune to one or more of the root-knot species, while severely attacked by the other species. Watermelons, for example, were not attacked by *M. hapla*, although severely attacked by the other species. Peanuts were not attacked by *M. incognita*, *M. incognita* var. *acrita*, or *M. javanica*, but are readily attacked by *M. hapla* and *M. arenaria*. Pepper seedlings are lightly attacked by *M. javanica* although very susceptible to the other four species. It is believed that these and similar host reactions will eventually make it possible to identify a given root-knot population by testing it against a few possible host plant species.

The wilting mechanism in Fusarium wilt of tomato. SCHEFFER, R. P. Critical examination was made of lycomarasmin in the production of wilt in tomato infected by *Fusarium oxysporum* f. *lycopersici*. If this toxin is the chief wilting agent, increased water loss would be expected immediately preceding or accompanying wilting. Instead, transpirational history of diseased plants showed a progressive decline in water loss as disease developed. The high toxicity of lycomarasmin to tomato cuttings depends on the presence of free iron. There was, however, no difference in disease development in inoculated iron-deficient plants, normal plants, and plants given excess iron. The comparative conducting ability of diseased and healthy plants was determined by placing roots of transpiring plants in dye solutions until vessels of healthy plants were well stained. Clearing the stems by various techniques showed that many vessels in diseased plants were not conducting. The same results were obtained when dyes were drawn through segments of stems and petioles. The reactions of plants in various stages of disease indicated that blocking was the immediate cause of wilt. Crude filtrates from cultures of the fungus and purified lycomarasmin caused injury but no plugging.

Pathogenic variation of Colletotrichum phomoides and related tomato fruit rot fungi. SCHMITTHENNER, A. F. Sixteen isolates of *Colletotrichum phomoides*, two isolates of *C. lagenarium*, and two isolates of *Glomerella cingulata* were screened for differences in pathogenicity which would be of value in an investigation of the physiology of parasitism. Three degrees of pathogenicity were indicated by rapid lesion development, slow lesion development, or absence of lesions in inoculated ripe tomato fruits. Thirty tomato accessions were surveyed, representing the species *Lycopersicon esculentum*, *L. pimpinellifolium*, *L. peruvianum*, *L. hirsutum*, and intermediate types. Eight isolates, four *C. phomoides* types, two *C. lagenarium* types, and two *G. cingulata* types were readily distinguishable as pathogenic races when only seven of the tomato accessions were used. These eight isolates were also distinct in appearance on potato-dextrose agar. The rates of growth of these same isolates on agar containing Seitz-filtered ripe-fruit juice from each of the seven tomato types did not correspond in every case to the rates of lesion development in the ripe tomato fruits. It is inferred that the pathogenic differences may be associated with either or both filterable and nonfilterable constituents of the fruits tested.

Host range of the sugar-beet root-rot fungus, Aphanomyces cochlioides Drechs. SCHNEIDER, C. L. Weeds, crop plants, and ornamentals representing 20 families were grown in soil naturally infested with *Aphanomyces cochlioides* and in steamed soil artificially infested with the fungus. Plants of the following families and species became infected: Chenopodiaceae: *Beta vulgaris* var. *cicla*, *B. patellaris*, *B. trigyna*, *Chenopodium album*, *Kochia childsi*, *K. scoparia*, *Salsola kali*, and *Spinacia oleracea*; Amaranthaceae: *Ameranthus blitoides*, *A. retroflexus*, *Celosia cristata*, and *Gomphrena blobosa*; Caryophyllaceae: *Dianthus heddewigi*. No difference in pathogenicity on sugar-beet and mangel seedlings was noted between monosporous cultures isolated from spinach and those isolated from sugar beets. A laboratory method permitting a rapid screening of plants for a host-range survey was adopted. Seedlings were placed in Petri dishes containing 20 ml. of a suspension of zoospores of *A. cochlioides*, and the laboratory results corresponded with those obtained when plants were grown in infested soil. Diagnostic symptoms appeared on susceptible plants within 3 days.

The production of zoospore inoculum of Aphanomyces. SCHNEIDER, C. L., AND H. G. JOHNSON. The following method has been adopted for obtaining zoospores for inoculum in testing the resistance of sugar beets to *Aphanomyces cochlioides* and the resistance of peas to *A. euteiches*. Cultures are grown for 5-7 days in flasks containing a sterile decoction of maize kernels or Pablum in tap water. The broth is then decanted, the cultures are rinsed twice, and the flasks are half-filled with sterile tap water. The cultures are kept sterile throughout the procedure. Within 12 hr. zoospores are produced, often more than 100,000 per ml. as determined with a counting chamber. After the spore suspension is decanted for use as inoculum water may again be added to the flasks to obtain additional crops of spores. Temperatures between 15° and 20° C. are optimum for production of zoospores. A suspension of approximately 100,000 zoospores per ml., poured into each 4-in. pot after the plants have emerged, is adequate inoculum for determining varietal differences in the resistance of sugar beets and peas.

Necrosis of sieve tubes below the bud unions of lemon trees on sour-orange rootstocks. SCHNEIDER, HENRY. The deterioration of lemon trees in California at an early age has long been a problem. It was early recognized that most lemon strains on sour-orange rootstock were especially subject to decline and as a result the use of that rootstock has been largely discontinued. Necrosis of sieve tubes and hyperplasia of rays immediately below the bud union of declining trees on sour-orange rootstock have been observed. Strains free of decline are free of necrosis below the union. Indications are that the strain of lemon and not the strain of rootstock is responsible for the decline. The Limoneira Company budded scions from a declining Lisbon lemon tree onto rootstocks propagated from the rootstock of the same declining parent tree. A study of this material revealed that necrosis occurred below the bud union of the reconstructed trees, whereas buds from a healthy Lisbon tree budded onto the rootstock propagated from the declining tree developed trees which did not show necrosis. Allen Eureka scions on a rootstock propagated from a healthy Lisbon tree caused necrosis below the bud union at 13 years. The necrosis appears to occur in cycles.

The importance of bud unions in the development of lemon sieve-tube necrosis. SCHNEIDER, HENRY. It was reported earlier that the necrosis of sieve tubes above the bud union of lemon trees can, when it becomes extensive and prolonged, cause a decline or a collapse of lemon trees by preventing translocation. The question arose as to whether necrosis farther up the trunks in lesser amounts resulted indirectly from the girdling (necrosis) just above the bud union or from the disorder itself. Eureka lemon trees propagated from cuttings exhibited considerable necrosis but not so much as was found immediately above the bud union of budded trees. These trees were growing near the coast. In a semicoastal area, only part of the Eureka trees propagated from cuttings exhibited necrosis, while Eureka trees at Riverside (inland) were free of it. A strain of Lisbon lemon propagated from cuttings and growing on the coast was free of necrosis; it was also free when on sweet-orange stock. Rootstocks provoking necrosis above the union but free of it themselves are sweet orange, sour orange, grapefruit, various mandarins, Palestine lime, and Sampson tangelo. Rough lemon stocks at times show some necrosis of older sieve tubes below the bud union. Lemon trees propagated on various lemon strains in some instances showed necrosis above and below the union. However, for two lemon strains used as stocks necrosis was present above the bud union but absent below.

Parasitism as related to physiologic specialization in Col-

letotrichum lini Bolley. SCHWINGHAMER, E. A. Isolates of *Colletotrichum lini* obtained from Minnesota flax samples differed markedly from one another in cultural characters and in virulence on flax varieties. Three race groups within the species have been differentiated on the basis of seedling reactions of six flax varieties: Biwing, Crystal, Punjab, Redwood, Renew, and C.I. 463. One race group is very virulent, one is moderately virulent, and the other is only weakly pathogenic. Pathogenicity is associated with spore germination, appressorium formation, and penetration. Virulent and moderately virulent isolates form numerous, normally shaped appressoria which can penetrate cellophane membranes and the epidermis of most flax varieties. Weakly pathogenic isolates comprise two types: 1) those which produce abundant, apparently normal appressoria that penetrate cellophane membranes and the host epidermis only infrequently, and 2) those which generally fail to produce appressoria on cellophane membranes but form elongated, irregularly shaped appressoria on the host surface. Abrasive wounding of flax seedlings before inoculation accelerated the infection of a susceptible variety by a weak isolate, but did not appreciably affect the natural physiologic resistance of a variety.

Volatile chemicals for the inhibition of fruit losses in storage and transit. SHARVELLE, ERIC G., AND J. S. VANDERMARK. Preliminary experiments on the control of storage and in-transit rots of perishable fruits by space fumigation with volatile hydrocarbons have given highly encouraging results. Development of Rhizopus rot in strawberries incubated for 24 hr. at room temperature after artificial inoculation was completely inhibited by a 24-hr. exposure to vapors of the volatile hydrocarbons, trichloroethylene, tetrachloroethylene, and s-tetrachloroethane at concentrations ranging from 1 part of chemical in 4000 parts of air to concentrations as low as 1:40,000. Brown rot of artificially inoculated apples incubated for 24 hr. prior to treatment was also completely inhibited by the same range of concentrations of these chemicals. Brown rot (*Monilinia fructicola*) has been completely inhibited at room temperature over a 4-day period following inoculaton when artificially inoculated peaches were exposed for 9 hr. to these volatile hydrocarbons. In-vitro tests demonstrated that these chemicals, at concentrations ranging from 1:4000 to 1:40,000, were lethal to pure cultures of *Monilinia fructicola*, *Rhizopus nigricans*, *Penicillium* sp., and *Bacterium* sp., grown on potato-dextrose agar.

Inheritance of cedar rust resistance in apple. SHAY, J. R., AND L. F. HOUGH. Resistance to cedar apple rust is present in certain commercial apple varieties. A few of these have been used as quality parents in the cooperative apple scab resistance program of the Illinois, Indiana, and New Jersey Agricultural Experiment Stations. Greenhouse-grown seedlings from controlled crosses which survived artificial epiphytotics of apple scab were subjected to cedar rust inoculations before removal from the greenhouse to the field in late May. As found by other workers, our preliminary data indicate that cedar rust resistance and susceptibility are controlled by a single major factor pair with resistance dominant. Inoculations of 1386 seedlings from crosses of McIntosh with 13 different susceptible seedlings yielded 1333 resistant and 53 susceptible seedlings. These data suggest that McIntosh is homozygous for the major gene pair but that other factors are present which may modify the expression of the factor for resistance. Each progeny of crosses of Delicious, Macoun, and Wolf River with susceptible seedlings could be divided into nearly equal resistant and susceptible classes. The data suggest that these three varieties are heterozygous for a single factor pair conditioning cedar rust resistance and susceptibility.

Interrelations of seed-treatment chemicals and commercial nodule bacteria inoculum as measured by yield of soybeans grown in steamed and nonsteamed soil. SHERF, ARDEN F. Soybean seed treated with Arasan, with and without the addition of nodule bacteria, were planted in steamed and nonsteamed soil in 6-in. pots in the greenhouse and also in 3-gal. crocks out-of-doors. Results of the two studies, including data on stand, foliage weight, and bean yield, are combined herein. Chemical seed treatment increased stands 17 per cent in nonsteamed soil and encouraged early emergence. Foliage and bean yields were increased about 25 per cent over the controls. In steamed soil no increases in these categories were evident. Nodule bacteria decreased foliage and bean yields when added to either nontreated (10 per cent decrease) or Arasan-treated seed (22 per cent decrease) in nonsteamed soil. In steamed soil the decrease was 16 per cent for foliage and 14 per cent for beans when bacteria were added to nontreated seed; no differences were noted when bacteria were added to Arasan-treated seed. Thus nodule bacteria under some conditions appear to be harmful. Since yields from the Arasan-plus-bacteria series were equal to those receiving only bacteria, Arasan evidently did not inhibit such bacteria in either soil series. Rank of treatments on the basis of foliage and bean yield was: Arasan, Arasan with bacteria, control, control with bacteria.

Seed treatment and inoculation studies on soybeans in Iowa fields, 1950-1951. SHERF, ARDEN F., AND C. S. REDDY. In field tests with soybeans using 51 replications in 10 locations, Arasan treatment gave mean stand increases of 9.6 per cent. In 21 replications in six locations Spergon gave a 10.8 per cent increase. Treatment with Arasan or Spergon gave increases of 1 bu. and 0.4 bu. per acre respectively over the controls. Addition of nodule bacteria to untreated seed resulted in an increased yield of only 0.3 bu. per acre over the noninoculated control. Yields from plots whose seed had been treated with Arasan or Spergon and which were also inoculated with nodule bacteria were equal to each other as well as to those from the inoculated control plots, thus indicating that no harmful effect of the chemicals on the bacteria need be feared in most soils. At two locations bean yields were increased by bacterial inoculation, but yields were reduced when either Arasan or Spergon was used in conjunction with the bacteria. Under conditions of these tests, in widely varying soil types with variable cropping histories, treatment of good quality soybean seed with Arasan or Spergon gave stand increases approximating 10 per cent; however, corresponding increases in yield did not follow. In most soils bacterial inoculation was not needed. In soil favorable for soybean growth, planted with good seed at 1 bu. per acre, neither treatment nor inoculation gave significant increases. On soils where bacteria are needed, chemical treatment should not be used.

Control of peach brown rot with the sodium salt of dehydroacetic acid. SHURTLEFF, M. C., AND V. G. SHUTAK. Ripe peaches of Summer Crest, South Haven, Early Elberta, Elberta, and Salburta varieties in lots of 10 each were inoculated by spraying of sandpapered surfaces, dipping of uninjured fruits, and pinpricking fruits. One-half hour after inoculation fruits were either dipped for 10 sec. in or sprayed with tap water, wettable sulfur suspension (8-100), or the sodium salt of dehydroacetic acid (0.5, 1.0, and 1.5 per cent). Peaches were stored at 21° C. ± 1° and 73 per cent ± 5 per cent relative humidity for 3–5 days, when development of rot was recorded. The sodium salt of dehydroacetic acid (DHA-S) (at 1.0 and 1.5 per cent) was far superior to wettable sulfur in preventing storage rot. For Elberta the relative percentage of rot developing was: water dip, 99.0; wettable sulfur, 79.5; and DHA-S, (0.5 per cent) 43.2, (1.0 per cent) 15.4, and (1.5 per cent) 3.1. Tree-sprayed ripe Salburta fruit developed the following percentage rot after 5 days: water spray, 35.3; wettable sulfur, 34.8; and DHA-S, (0.5 per cent) 7.0, (1.0 per cent) 1.6, (1.5 per cent) 0.0. DHA-S did not injure the foliage or affect the appearance, odor, or taste of the peaches.

Electron microscopy of cucumber virus 1. SILL, W. H., JR., W. C. BURGER, M. A. STAHMANN, AND J. C. WALKER. Electron photomicrographs indicate that cucumber virus 1 may be a spherical particle. These particles were generally aggregated and were approximately 350 Å in diameter. Extracts from healthy plants were noninfective and showed no such particles. Preparations from leaf tissue of infected tobacco and cucumber did not give good electron photo-

micrographs, but satisfactory ones were obtained from extracts of infected cucumber corollas. This observation, plus the fact that all the infectivity in leaf extracts, as measured by local lesions on cowpea, var. Black, was sedimented at low speed (5000 × G.) whereas the infective corolla preparations required high speeds (36,000 × G.) for complete sedimentation, suggests that the virus in the leaf must be highly aggregated or strongly adsorbed to leaf tissue components. A consistent relationship was noted between infectivity and the presence of chlorophyll in differentially centrifuged leaf fractions. These observations plus the fact that preparations of chloroplasts from infected tissue were shown to contain a large fraction of the virus infectivity suggest again that the virus combines with some leaf tissue components. The sedimentation of the virus from infected corolla extracts over a wide range of speeds supports the view that it also aggregates readily.

Purple and brown blotch of onions. SKILES, ROBERT L. Both purple and brown blotch of onions in the Arkansas Valley of Colorado may be caused by *Alternaria porri*, *A. tenuis*, or *A. tenuissima*. Leaves of Colorado 6 strain of Yellow Sweet Spanish, and bulbs of Colorado 6 and Ferry-Morse selection of Utah strain of Yellow Sweet Spanish were inoculated. The *A. porri* isolates were generally the most pathogenic on leaves and bulbs. Distinct physiologic races were evident within each of the fungus species. Racial differences were found within *A. tenuis* on Colorado 6 bulbs, within *A. tenuis* and *A. tenuissima* on mechanically injured Colorado 6 leaves, and within all three species on uninjured leaves of the same strain. One isolate of *A. tenuissima* was more pathogenic on uninjured leaves than were several of the *A. porri* isolates. Morphologic, physicochemical, and cultural characters of the physiologic races within each species could not be correlated with pathogenicity.

Aphid transmission of strawberry viruses from commercial plants to Fragaria vesca L. (East Malling clone). SMITH, HARLAN E. Virus-free wingless *Capitophorus fragaefolii* Ckl. were allowed a 3-hour and a 6-day acquisition feeding on each of three selected commercial plants. The aphids from each feeding on each plant were separately transferred for infection feedings on successive healthy plants of *F. vesca* for the following intervals in hours: 1, 23, 24, 24, and 24, then for 48-hr. intervals until all aphids died. Virus 2 (Demaree and Marcus) was transmitted after the 6-day acquisition feeding followed by the 23-hr. infection interval, and persisted in the vector 9 days. Optimum air temperature for symptom expression was 24°–28° C. Symptoms occurred in 23 days. Virus 1 (Demaree and Marcus) was transmitted after the 3-hr. acquisition feeding followed by the 1-hr. or 23-hr. infection feeding interval. Symptoms occurred in 10 days. The isolate from the third plant caused dwarfing, extreme arching of midvein, and irregular chlorosis along veins. Later, irregular green areas remained between veinlets. Symptom expression seemed to be dependent on cool air temperature. This isolate persisted in the vector up to the first 24-hr. interval after the 6-day acquisition feeding, but the aphids did not become infective after the 3-hr. acquisition feeding.

Dodder transmission of strawberry viruses. SMITH, HARLAN E., AND J. DUAIN MOORE. When stems of dodder (*Cuscuta sub-inclusa*) were trained from healthy to selected diseased strawberry plants and thence to healthy *Fragaria vesca* (East Malling clone) for 2 weeks, virus 1 (Demaree and Marcus) was transmitted with 26 per cent infection and symptoms in 11–17 days, and virus 2 (Demaree and Marcus) with 46 per cent infection and symptoms in 25–40 days. A third isolate, which caused severe "shock" symptoms (leaf downcurling, irregular chlorosis, and dwarfing) and later became masked, was transmitted with 33 per cent infection; symptoms occurred in 28-38 days. All isolates were derived from different plants. Infection with virus 1 was increased to 75 per cent when the inoculated plants were darkened for 3 days, beginning when the dodder was first placed on them. Virus 1 also was transmitted with *Cuscuta gronovii*. Seventy-five per cent of *Asclepias syriaca* plants had irregular chlorotic areas, sometimes followed by general chlorosis, necrosis, and defoliation, when inoculated from a virus 1 type strawberry plant through *Cuscuta sub-inclusa*. Symptoms occurred in 14–23 days and were reproduced by grafting diseased milkweed to healthy milkweed plants. Dodder transmission attempts from diseased milkweed to indicator plants failed to produce symptoms of virus 1 type.

Pythium and other fungi colonizing sterilized straw in the soil. STAFFELDT, E. E., AND G. SEMENIUK. Sterilized oat and clover straws were buried in Clarion loam soil and the fungi colonizing them within 1, 2, 4, 8, 16, and 32 days were determined. In the first of two trials, one moisture level and seven temperatures were used; in the second, three moisture levels and four temperatures were used. In both trials the straws were cleaned without surface sterilization and planted directly on a carrot agar medium in Petri dishes. In these tests, fungi colonized the straws within one day at all temperatures and soil moistures. Representatives of *Pythium*, *Rhizopus*, *Rhizoctonia*, *Alternaria*, *Fusarium*, *Phoma*, *Chaetomium*, *Aspergillus*, *Penicillium*, and *Stachybotrys* predominated in the first trial; in the second, species of *Trichoderma*, *Myrothecium*, *Sporotrichum*, and *Verticillium* were added. A chlamydospore-producing fungus was prevalent in both trials. Different fungi colonized the straws at different rates in response to temperature and moisture. *Pythium paroecandrum*, *P. irregulare*, *P. acanthicum*, and nonoosporeforming spherical sporangiate Pythium species were the predominant pythiaceous forms colonizing the straws.

Studies on an antifungal antibiotic produced by Bacillus subtilis. STESSEL, G. J., CURT LEBEN, AND G. W. KEITT. An antibiotic, tentatively designated toximycin, was obtained in a partly purified form on fractionation of shaken corn-steep cultures of *B. subtilis*, soil isolate A67. The *Glomerella cingulata* agar diffusion assay was used to estimate activity. Yields were up to 250 mgm. per liter, or about 50 per cent recovery. The partly purified material completely inhibited 20 fungi and three bacteria at concentrations from 6.25 to 100 μgm. per ml. in agar-streak tests, and in *in vitro* tests inactivated the tobacco necrosis virus. In protectant studies in the greenhouse with *Alternaria solani* on tomato foliage, the LD50 and the LD95 were, respectively, 45 and 300 μgm. per ml. Tomato cuttings took up to 70 units (equivalent to 17.5 mgm.) per cutting from antibiotic solutions; above 2.5 units wilting occurred. Wilted cuttings recovered on transfer to water, but subsequent water uptake was reduced. Root length and number were markedly increased on cuttings in antibiotic solutions. The partly purified material was nontoxic to five species of plants when applied as a spray.

Life histories of the root-knot nematodes. TARJAN, A. C. Comparative studies of five root-knot nematodes (formerly *Heterodera marioni*, now *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. incognita* var. *acrita*, and *M. javanica*) were made with seedlings of snapdragon, *Antirrhinum majus* hort. var. Margaret, and tomato, *Lycopersicon esculentum* hort. var. Rutgers, as hosts. On snapdragon *M. arenaria*, *M. hapla*, and *M. incognita* oviposited 30 days after plants were inoculated. Reinfection of root tips by second-generation larvae was detected 66 days after inoculation with *M. arenaria* and 68 days after inoculation with *M. hapla*. On tomato *M. incognita* var. *acrita* formed eggs in 37 days, whereas the other species required 39 days for oviposition. Reinfection of root tips by second-generation larvae occurred 57 days after inoculation with *M. incognita*, 59 days after inoculation with *M. incognita* var. *acrita*, and 63 days after inoculation with *M. arenaria*, *M. hapla*, and *M. javanica*. Significant differences between species in the time required to attain various stages of development were not found, whereas developmental intervals did differ markedly for the hosts used.

Stalk-rot development in corn following the European corn borer. TAYLOR, GORDON S. *Cephalosporium acremonium*, *Fusarium moniliforme*, bacteria and yeasts generally, *Gibberella zeae*, *Nigrospora oryzae* and *Mucor* species oc-

casionally, and *Diplodia zeae* rarely, were associated with a rot produced in corn stalks following entrance of the European corn borer. Inoculation of corn stalks with these organisms by toothpick and hypodermic methods resulted in various degrees of rot development. Bacteria, *Cephalosporium acremonium*, and *Fusarium moniliforme* were often reisolated from the rotted tissue along with the introduced organism. Simultaneous inoculation with *Diplodia zeae* and *Gibberella zeae* or *Fusarium moniliforme* in the same wound resulted in rot development approaching that of *Diplodia zeae* alone, but when these fungi were introduced at different times the extent of rotting approached that caused alone by the organism first introduced. More rot was produced when *Gibberella zeae* and *Fusarium moniliforme* were introduced simultaneously than when alone, and generally more rot was produced when *Fusarium moniliforme* preceded *Gibberella zeae* than in the reverse situation. The retarded development of *Diplodia zeae* in wounded tissue previously infected by other organisms may account for the infrequent isolation of this fungus from rotted tissues around corn borer tunnels.

Effect of cycloheximide (actidione) and some environmental factors on the germination of spores of Monilinia fructicola and Botrytis cinerea. THANOS, ANDREW. *In vitro* tests were conducted to determine the effect of actidione under various microenvironmental conditions. Spore suspensions, incorporating various concentrations of actidione, were prepared and studied under a series of temperatures ranging from 22° to 30° C. Additional tests were made to determine the effect of pH at each of the above temperatures. Actidione was most effective in inhibiting germination of *M. fructicola* at 26° C. At this temperature inhibition of germination was first noted at 2.5 p.p.m. and gradually increased until at 10 p.p.m. no germination occurred even after 72 hr. The optimum pH at this temperature was 4.0. An experiment was also prepared to determine what effect actidione might have, when in contact with the spores of *M. fructicola* in the dry state, over a period of time from 1 to 12 months. In the dry state actidione was not significantly different from the above tests. *Botrytis cinerea* was erratic in its response to actidione. At 10 p.p.m. it was significantly inhibited as compared to the control, while at 25 p.p.m. a stimulation of germination was noted which rapidly decreased at higher concentrations until no germination occurred at 50 p.p.m.; these results were obtained at 22° C. at pH 6.80.

Varietal susceptibility in safflower to Phytophthora root rot. THOMAS, C. A. Root rot, caused by *Phytophthora drechsleri*, has developed as one of the more important diseases of safflower, *Carthamus tinctorius*, in the western part of the Great Plains and in California. Marked differences in susceptibility have been noted in the field among present varieties, selections, and foreign introductions. Varieties Nebraska 9, N852, and Indian are susceptible, N6 is intermediate, and N8, N5, and N4 are resistant. A greenhouse method of evaluating resistance was developed as an aid to breeding programs and studies on inheritance of resistance. The method consisted of inoculating 1- or 2-week-old plants with a mycelial suspension at a soil temperature of 25° to 30° C. Age of plants when inoculated and soil temperature were important in obtaining a good differential between resistant and susceptible types. High soil moisture favored disease development but was not so important as temperature and age for desired results.

Symptomless carriers of Fusarium in the carnation. THOMAS, W. D., JR., AND R. R. BAKER. Fusarium wilt often develops in the Virginia variety of carnation when cuttings from supposedly healthy mother plants are propagated. Six per cent of isolations made from 20 carnation varieties showing no symptoms of wilt yielded *Fusarium oxysporum* f. *dianthi*. Of the isolations made, 81 per cent from Virginia, 10 per cent from White Patrician, 36 per cent from Pink Patrician, 16 per cent from Starlight, and 13 per cent from Northland gave rise to *Fusarium*. Trace amounts of the fungus were obtained from Frosted Pink Patrician, Light Pink Patrician, Louise, and Sylvia. After 4 months no symptoms were evident on parent plants. Tests on healthy indexed White Patrician with the isolates obtained indicated at least four degrees of pathogenicity. The carrier phenomenon was most pronounced in established plants, the presence of the pathogen becoming evident primarily when cuttings from these plants were propagated. Variability of symptom expression was due to the presence of biotypes of the fungus and to the effect of environmental factors.

Root transmission of carnation mosaic virus. THOMAS, W. D., JR., AND R. R. BAKER. The pattern of spread of carnation mosaic to adjacent plants has given rise to speculation as to the possibility of root transmission of the virus. Mosaic-free plants of *Dianthus barbatus* were planted in pots together with mosaic-infected carnation plants. To prevent other sources of infection, the plants were separated by glass plates, and the soil covered with paraffin. A similar series using noninfected carnations served as a check. Within two months all *D. barbatus* growing adjacent to infected carnations had developed mosaic symptoms. No symptoms were evident on test plants adjacent to mosaic-free carnations. Examination of the roots indicated many natural grafts between the plants. Repeated tests in insect-proof cages showed that the carnation mosaic virus may be transmitted through roots. There was no evidence that the virus was carried from infected plants by the soil solution.

Laboratory culture of the rice white tip nematode, and inoculation studies. TODD, E. H., AND J. G. ATKINS, JR. A method was developed for artificial culture of the rice white tip nematode, *Aphelenchoides oryzae* (?). Several nematode-infested rice grains were placed in flasks of steamed, unhulled rice following washing in mercuric chloride and sterile water. The seed germinated but the seedlings soon died. When fungi, generally species of *Helminthosporium* or *Fusarium*, were present and grew on the rice substrate, large numbers of nematodes were present after a few weeks. In the absence of fungi the nematodes failed to multiply. Inoculation studies were conducted using hot-water-treated seed of susceptible varieties planted in crocks of steamed soil. Inoculated plants developed typical leaf and panicle symptoms. Control plants were disease-free. Syringe and soil inoculations were both used successfully. Pure cultures of *Fusarium* sp. from the nematode cultures failed to induce white tip symptoms. Nematodes were found associated with inoculated plants that had typical symptoms. Nematode cultures were obtained from seed of inoculated plants. With modifications, Koch's rules of proof have been satisfied for the white tip disease. Small tests have established the value of field inoculations with artificial cultures in the rice breeding program for white-tip-resistant varieties.

Blister-shake, a weather-induced defect in yellow poplar. TRUE, R. P., AND E. H. TRYON. Narrow tangential cavities between 1935 and 1936 growth rings of yellow poplar, chiefly on the south and west sides of trunks, were found in 1950. In 1936 the diameter of these stems had been about 6 in. at stump height. The cavities partially or completely encircled the boles and extended upward 4–11 ft. A 1936 frost ring completed the encirclement of trunks only partly encircled by a cavity. Wound wood made up the inner part of the 1936 ring where the shakelike defect occurred. The 1936 ring was sharply narrowed by indentation at the limits of the separation and bent somewhat outward from the 1935 ring to cover and enclose the cavity between them. This separation seems to have been, in the spring of 1936, a large blister whose base was the 1935 annual ring and whose covering was then the bark and adhering cambium. The cambium, transposed but living, laid down woody tissues, converting the blister cavity into a permanent shakelike defect. A late frost with alternate freezing and thawing following the severe winter of 1935–1936 appears to have caused the condition.

The evolution of susceptibility to tobacco mosaic in Nicotiana and the origin of the tobacco mosaic virus. VALLEAU, W. D. Plantago mosaic virus (PMV) is widely distributed

(in North America at least) in *Plantago* sp., and is frequently found in tobacco when the plant bed is placed in a dense stand of plantain. PMV withstands drying and is adapted to surviving from year to year in perennial plantains and in cured tobacco. The numerous strains of tobacco mosaic virus (TMV) could all have resulted from mutations of PMV, and may have appeared as a pathogen of *Nicotiana* only after cultivation of *N. tabacum* was begun. The following theory on the evolution of *Nicotiana* species is advanced: The progenitor was a plant resistant to TMV with the genetic constitution *NN* (hypersensitivity to TMV) and *AA* (susceptibility to tobacco mosaic). Mutation from the dominant *N* to *N'* would result in species that react necrotically to PMV and certain strains of TMV, and by mottling to other strains of TMV; mutation to *n* would produce species which react by mottling to PMV and to all strains of TMV. According to this view, TMV was not associated with *Nicotiana* during its evolution, and dominant resistant mutants were not encouraged by association of susceptible species with TMV as postulated by Holmes.

Mechanical transmission of a virus from mosaic-infected elm to herbaceous plants. VARNEY, E. H., AND J. DUAIN MOORE. A ringspot-type virus was transmitted mechanically to Havana tobacco from immature American elm leaves with mosaic symptoms. Necrotic spots and concentric rings appeared in 2–5 days after inoculation; leaves of systemically infected tobacco showed ringspots, oak-leaf patterns, mottle, or no symptoms. The virus was recovered from tobacco roots, leaves, calyces, corollas, ovaries, and the styles and stamens. On cowpea, stem necrosis developed in 5–7 days; no local lesions were observed. Symptoms on garden pea consisted of a faint mottle and veinclearing. Inoculated cotyledons of cucumber had chlorotic lesions; subsequent leaves had chlorotic spotting, mosaic, or no symptoms. One trifoliate leaf on 1 of 7 inoculated garden beans developed necrotic flecks; inoculated simple leaves remained symptomless. Recovery to tobacco was made from all hosts with symptoms as well as from two symptomless hosts, *Nepeta cataria* and *Physalis heterophylla*. One of three elm seedlings mechanically inoculated with juice from diseased elm leaves had chlorotic spots on an inoculated leaf. The next four or five new leaves were stunted and had faint mottling; still later leaves were nearer normal size with no symptoms. No infection of elm seedlings was obtained following inoculation with extracts from diseased cowpea or tobacco plants. Controls remained virus-free.

Comparison of cycloheximide (actidione) with ten other fungicides for the control of turf diseases. VAUGHN, JOHN R., AND WILLIAM KLOMPARENS. Previous work by the authors has shown that cycloheximide (actidione), an antibiotic produced by *Streptomyces griseus*, is an effective fungicide for certain diseases of fine turf. In 1951 this material was compared with ten other fungicides in a replicated plot of Seaside bentgrass turf located on the College farm at East Lansing. The area was uniformly inoculated with four turf disease fungi, and heavy infection was obtained with the dollar-spot pathogen (*Sclerotinia homeocarpa*). Excellent control of dollar spot was obtained in the plots sprayed with cycloheximide (actidione), two chromate compounds (Crag 1025 and Crag 531), and an organic cadmium compound (Cadminate). Good control was obtained in the plots sprayed with inorganic mercury (Calo-clor) and an organic sulfur compound (Vancide 51), while fair control was accomplished by use of a phenyl mercury compound (Tat-C-lect), a mixture of hydroxymercurichlorophenol and hydroxymercuricresol (Special Semesan) and by thiram (Tersan). In another location, on large turf plots sprayed with cycloheximide or with phenyl mercury, the cycloheximide was completely effective for the control of melting-out caused by *Helminthosporium* sp. while the phenyl mercury did not check this disease at all.

Effect of stunting agents, Fusarium lycopersici and maleic hydrazide, upon phosphorus distribution in tomato. WAGGONER, PAUL E., AND A. E. DIMOND. Tomato plants were inoculated with *Fusarium* and/or sprayed with maleic hydrazide (MH). Plants were fertilized with radiophosphate 11 or 13 days later and were harvested on the 14th day. P^{32} contents (per mgm. of tissue) were compared between the four treatments and these results were related to severity of vascular discoloration. MH alone caused no vascular discoloration, reduced plant weights to one-sixth of check weights, and lowered P^{32} content of stems and, especially, of leaves and growing points. *Fusarium* alone caused vascular discoloration, reduced plant weights to one-half, and did not significantly alter amount or distribution of P^{32}. Effect of *Fusarium* was more severe in plants whose phosphorus contents were decreased by treatment with MH. MH is inferred to stunt by suppressing metabolism and, hence, phosphorus content. In contrast, *Fusarium* is inferred to stunt by consuming food and nutrients and by producing toxins. Consequently, metabolism and phosphorus content of combined host and parasite are not suppressed. These inferences lead to a mathematical model relating quantity of *Fusarium* and phosphorus to growth of host. The model is consistent with other facts concerning behavior of *Fusarium*. Atomic Energy Commission support is acknowledged.

Studies on the inheritance of resistance to downy mildew in onion incited by Peronospora destructor. WARID, W. A., AND E. C. TIMS. In 1948, F_1 seeds were obtained from crosses made by the U. S. Department of Agriculture between plants of the Calred and Creole varieties of onion. Calred possesses moderately resistant foliage and highly resistant scapes (seedstalks), whereas the foliage and scapes of Creole are highly susceptible to mildew. The progenies were tested in the field in the 1949-1950 and 1950-1951 seasons. There was a natural epiphytotic of mildew the first season. The 1950-1951 season was dry, but the disease was present and spread by an artificial epiphytotic. In the F_1 plants both foliage and scapes were susceptible. In 14 F_2 populations, totaling 5142 plants, 2.8 to 24.2 per cent of the plants showed moderate foliage resistance. The degree of scape infection was recorded for 11 F_2 populations. Segregation appeared to fit a 15:1 ratio in six populations, and a 3:1 ratio in four, with susceptible scapes dominant. In one F_2 population, all plants had susceptible scapes. The assumption is made that the Calred plants possessed one or two genes for resistance. These genes, tentatively designated s_1 and s_2, were independently inherited and formed a series of duplicate genes, each recessive to its allele for susceptibility, S_1 and S_2. There was no true association between the degree of foliage infection and that of scape infection, the r value being 0.11.

Effectiveness of seed treatment in controlling damping-off in alfalfa. WEBER, PAUL V. V. The effectiveness of Arasan, Dow 9B, Phygon XL, and Spergon applied at two rates as seed treatments on clean, certified Buffalo alfalfa was tested in noninfested and in differently infested sterilized soil in concurrent greenhouse trials. An infestation with *Colletotrichum trifolii* (three strains mixed) resulted in severe post-emergence damping-off which was not controlled by any of the seed treatments. Two infestations, one with a single Rhizoctonia isolate and one with a mixture of two Rhizoctonia isolates and *R. solani*, produced chiefly pre-emergence damping-off. All seed treatments were ineffective against the first of these; however, in the second, total emergence and stand were significantly improved by almost all. Finally, in the noninfested soil (where considerable post-emergence damping-off occurred) stand only was significantly improved by most of the seed treatments. These results suggest that similar variations may partly explain inconsistent field results.

Tall fescue seed can carry net blotch fungus. WELLS, HOMER D., AND J. LEWIS ALLISON. An epiphytotic of net blotch caused by *Helminthosporium dictyoides* produced severe leaf blighting and some lowering of seedling stands of tall fescue strains, *Festuca arundinacea*, in nursery plantings at Raleigh, North Carolina, during the fall of 1950. As this nursery was well isolated from other tall fescue plantings it was suspected that the initial inoculum had been introduced on seeds of one or all of the strains in the test; however, no difference in susceptibility to net

blotch was apparent among the strains. To determine whether or not the fungus may be seed-borne, seed lots of two southeastern-grown strains, Ky-31 and NC-211, and one western-grown strain, Oregon-Alta, were surface-sterilized in 70 per cent alcohol followed by a 3-min. dip in 10 per cent Clorox and plated on potato-dextrose agar. *H. dictyoides* was obtained from 3 to 5 per cent of all viable seeds of both southeastern-grown lots but it was not obtained from seeds of the Oregon lot. Typical net blotch symptoms were produced on seedlings of all strains when they were inoculated with a spore suspension of each of the above isolates of *H. dictyoides* under controlled conditions. These results indicate that *H. dictyoides* may be seed-borne on seed produced in the Southeast where the disease is omnipresent, and that seed-borne inoculum may account for epiphytotic build-up on plants from new seedlings.

Single spores and other fungus structures tagged with radiocarbon. WHEELER, H. E. Single spores and other fungus structures were effectively tagged by the use of C^{14}-labeled sucrose as the sole carbon source in the culture medium. The growth characteristics of four species of fungi, cultured on media having specific activities of 2.5, 5, 10, 20, and 30 microcuries per ml. remained unchanged except for retardation of the growth of one species at activities above 10 microcuries per ml. Fungi grown on radioactive media for 5 days (the period normally used for tagging) exhibited no mutagenic or lethal effects, but such effects were produced after much longer periods of growth. Tagged single spores and bits of mycelium were thoroughly washed and transferred to nonradioactive media and killed and autographed after periods of growth ranging from a few minutes to 48 hr. Calculations based upon the energy of the beta emission of C^{14}, the range of these particles in photographic emulsions, and measurements of the autoradiographic images produced indicated that no diffusion of C^{14} into the surrounding medium occurred during the growth of tagged fungi on nonradioactive media. These results enhance the possibility of detecting extracellular enzyme activity and the production of toxins in infection studies with tagged plant pathogens.

Ringspot of winter squash caused by the tomato ringspot virus. WILKINSON, R. E. Hubbard and Delicious type squash (*Cucurbita maxima*) are sometimes affected with a virus disease which produces ringspots on the fruit. The concentric rings extend various depths into the flesh and are actually concentric hemispheres or spheres delimited by water-soaked or corky boundaries. During storage checks or cracks develop in the rind of the fruit as the ringspots or spheres near the surface collapse and dry out. Such fruits soon rot because of the entry of *Fusarium* sp. and other fungi. The tomato ringspot virus (*Annulus zonatus*) can be isolated readily from fruits with ringspots. These symptoms have been reproduced in the field by inoculating young Hubbard squash vines with the virus. The tomato ringspot virus apparently is much more widely distributed in nature than has been recognized previously. The virus also was isolated from naturally infected plants of *Arctium lappa*, *Althaea rosea*, *Oenothera* sp., *Nicotiana alata*, *Solanum carolinense*, *Pastinaca sativa*, and *Pisum sativum*. All virus isolates were identified by reciprocal cross-protection tests with a known strain of the tomato ringspot virus. Protection was not obtained in comparable tests with a strain of tobacco ringspot virus (*Annulus tabaci*).

Parsnip canker is caused by Itersonilia sp. WILKINSON, R. E. Parsnip canker, a usually dry chocolate-brown rot, frequently develops near the shoulder or crown of parsnip roots late in the season and in storage. Although the disease has received more attention in England than in the United States, growers in New York and neighboring States consider it the main problem in parsnip production. A species of *Itersonilia* Derx has been shown to cause parsnip canker. The pathogen, presumably a lower basidiomycete, is sometimes difficult to isolate from cankers by the usual method of tissue plantings. It can be isolated readily, however, by spore shootings from infected tissue suspended above agar. The fungus also causes a leafspot, which apparently is the source of inoculum for the root rot or canker phase of the disease. Typical symptoms of the canker and leafspot phases were produced by inoculation of roots and leaves with pure cultures, and the pathogen was reisolated from the lesions produced. In 1950, a cool damp season, the leafspot was so severe in low areas of many fields that the plants were defoliated by mid-September. The leafspot also was prevalent on wild parsnips.

Comparative effectiveness in control of early blight on potato and tomato of differently formulated dithiocarbamates. WILSON, J. D. Zinc dimethyl dithiocarbamate (ziram), commonly used as a wettable powder, was found to give more effective control of early blight on potato and tomato when prepared from a slurry, and also when formed in the spray tank. Modifications of ziram in the form of zinc dimethyl dithiocarbamate cyclohexylamine (Zac) and as a half-and-half mixture of ziram and zinc mercaptobenzothiazole (Vancide 51 plus $ZnSO_4$) were also more effective when prepared in the spray tank than when used as the reaction products in the form of wettable powders. Likewise, tank-mix zineb usually has given slightly better results than zineb used as a wettable powder in the control of early blight. However, manganese ethylene bis dithiocarbamate gave better results when used as a wettable powder (prepared by the manufacturer) than as a spray tank mixture. Chemical opinion seems to be that conditions cannot be carefully enough controlled under casual field operations to insure a uniform product which will always be of maximum effectiveness in disease control.

Evaluation of resistance in tomato to Pseudomonas solanacearum by artificial inoculation methods. WINSTEAD, N. N. When resistant plants were inoculated by pouring a bacterial suspension over severed roots, disease resistance increased as the age of plants increased. In the susceptible Marglobe variety there was no decrease in disease incidence with increase in age, provided the plants were succulent when inoculated. The resistant tomato lines tested showed a greater degree of resistance in the roots than in the stems. In susceptible varieties either root or stem inoculations induced a high disease incidence. A high incidence of wilt occurred when as many as 450 susceptible young plants grown in rows in a flat 14 by 21 by 4 in. were inoculated by pouring a bacterial suspension over the severed roots. Disease incidence in susceptible plants was equally high following inoculation with dilutions up to 1:128 of an original standard bacterial suspension. For resistant lines disease incidence decreased as inoculum concentration was decreased. The causal bacteria were isolated from most plants of resistant lines which had no external symptoms of the disease.

Fungicides, timing of sprays, and methods of application for raspberry anthracnose control. WINTER, H. F. An experiment was conducted in 1951 to determine the effectiveness of various fungicides as "in foliage" sprays for raspberry anthracnose control. The fungicides used and dosages, in lb. per 100 gal., were as follows: ferbam, 2; manganese ethylene bis dithiocarbamate (70 per cent), 2; Orthocide 406, 2; Crag 341, 3 pt.; ferbam, 1, plus Phygon, ½. These materials were applied at prebloom and postbloom on New Logan plants which had received the standard delayed dormant spray of 8 gal. of liquid lime-sulfur per 100 gal. of spray. The results, given as average number of lesions per cane on July 20, were as follows: ferbam, 1.6; manganese ethylene bis dithiocarbamate, 2.9; Orthocide 406, 2.1; Crag 341, 11.5; ferbam plus Phygon, 2.5. Control, delayed dormant only, 25; no delayed dormant or in foliage sprays, 78. In the timing experiment the following results were obtained (lesions per cane): delayed dormant only (L.L.S. 8 gal.), 25; delayed dormant plus prebloom (ferbam 2 lb.), 8; delayed dormant plus prebloom and postbloom (ferbam 2 lb.), 1.6; prebloom and postbloom (ferbam 2 lb.) with no delayed dormant spray, 13. In a second experiment all of the above named fungicides were applied as 5× concentrations with an orchard con-

centrate sprayer at 1/5 normal gallonage. The results obtained were in all respects comparable to those obtained with dilute sprays. No discernible injury resulted from any of the sprays.

Production of indole acetic acid by Ustilago zeae, and its possible significance in tumor formation. WOLF, FREDERICK T. Indole acetic acid has been identified as a metabolic product of *Ustilago zeae*, and is present in culture filtrates. The identification of this compound is based upon positive tests with Salkowski, nitrite, and perchloric acid reagents, and upon identity of R_F values obtained on paper chromatography of filtrates with those provided by an authentic sample of indole acetic acid. Indole is not produced by *U. zeae*. Tests with gelatin, casein hydrolysate, and a number of individual amino acids indicate that tryptophane is the precursor of indole acetic acid. The mechanism of conversion of tryptophane to indole acetic acid is not known with certainty, but tryptamine has been excluded as a possible intermediate. These findings are interpreted as indicating that the development of tumors characteristic of corn smut disease may be due, at least in part, to the production of indole acetic acid by the pathogen.

Treatments to prevent fruit storage rots. YOUNG, WILLIAM J., AND E. S. BENEKE. Treatment in the form of dips and volatile gas was tried in 1951 in an attempt to control storage rots in raspberries, strawberries, and cherries. Compounds used for dipping were the sodium salt of dehydroacetic acid in 0.5 per cent solution, and actidione at 2 p.p.m. Fruits were also exposed to "Sound Delivery" gas, a proprietary product. Results showed DHA-S excellent in controlling the common storage molds, *Rhizopus*, *Botrytis*, and *Alternaria*, and in preserving the appearance of fruit. Actidione was of no value in controlling mold or preserving appearance. "Sound Delivery" provided only fair mold control, and it caused bleeding of cherries and raspberries.

A new necrotic-lesion-producing strain of yellow bean mosaic. ZAUMEYER, W. J. From distorted bean pods collected in eastern Washington in 1950, a new variant of yellow bean mosaic virus was isolated. In addition to producing necrotic lesions on the inoculated leaves of certain varieties, the virus produces mottle symptoms on the upper leaves similar to, but slightly more intense than, those caused by typical yellow bean mosaic. The necrotic spots when first formed are similar to those of pod mottle and Southern bean mosaic. Later they become dark red and increase to 1–2 mm. in diameter. Finally, their centers become gray and papery. Considerable veinal necrosis occurs, particularly on the underside of affected leaves. When the lesions are numerous and coalesce with others they form large scaldlike areas. Leaves thus affected soon fall. Other hosts on which local infection was obtained include tobacco, *Nicotiana rustica*, and *Vicia faba*. Systemic infection was obtained on white sweet clover. Thus far infection has not been obtained on alfalfa, crimson clover, red clover, white clover, pea, cowpea, or soybean. The virus is not seed-borne in bean. Its physical properties are comparable with those of typical yellow bean mosaic virus. The viruses of common bean mosaic, New York 15 mosaic, and yellow bean mosaic partially protect against this new variant.

Evaluation of soil fungicides for control of Phytophthora cinnamomi. ZENTMYER, GEORGE A. The need for an effective nonphytotoxic soil fungicide is obvious. A rapid laboratory screening test was developed that gives good correlation with results in greenhouse and field. Chemicals are applied to soil in shell vials, with soil type, moisture, temperature, and inoculum standardized. Minimum requirements: penetration of 1 in. of soil in concentration sufficient to kill the test fungus when the chemical was applied as a surface drench at a dilution of 1:500. Three of 40 fungicides passed the screening test: Dithane D-14 and Parzate liquid (nabam), Vancide 51, and Dowicide G. All other commercial fungicides tested were ineffective, including the organic mercurials, insoluble dithiocarbamates, quinones, hydroxy-quinolines, soluble quaternary ammonium compounds, copper sulfate, Orthocide 406, Crag 658, Karathane, and Dowicides A, B, and C. Dithane D-14 was the most effective soil fungicide tested, killing the test fungus (*Phytophthora cinnamomi*, root pathogen of many woody hosts) at a dilution of 1:2500 (1:13,150 active). D-14 drenches (1:500) reduced the population of *Phytophthora* with no injury to avocado seedlings in the greenhouse; 1:250 eliminated the fungus but was slightly phytotoxic. Monthly and bimonthly irrigations of avocado trees in the field with Dithane D-14 during the past two seasons markedly reduced the population of *Phytophthora:* it increased in the intervals between treatments but remained consistently lower than that of controls.

A substance stimulating sexual reproduction in Phytophthora cinnamomi. ZENTMYER, GEORGE A. A chemical present in feeder roots of Mexican avocado stimulates the production of the sexual stage by *Phytophthora cinnamomi*, a fungus that rarely produces oospores in culture. Roots were sterilized with propylene oxide, then extracted with cold or hot water. Oogonia and amphigynous antheridia formed in abundance in numerous tests in 2–3 days after inoculation of the aqueous extract (pH 6.5) with the fungus; oospores were evident in 4–5 days. The stimulatory substance is nonproteinaceous, withstanding autoclaving (15 lb. for 20 min.) and boiling (1 hr. at 99°C.); it is soluble in cold and hot water, removed from the aqueous extract by chloroform and ether, adsorbed on norit, and dialyzable. The homothallic, hermaphroditic nature of the fungus was demonstrated by production of both antheridia and oogonia in single zoospore cultures. Oospores were formed in the extract by isolates of *P. cinnamomi* from camellia, chestnut, cinchona, heather, pine, pineapple, rhododendron, and avocado (avocado isolates from California, Texas, Peru, Costa Rica, Honduras, and Mexico). Similar extracts of roots of other hosts of *P. cinnamomi* (chestnut, heather, pine) did not stimulate oospore formation. The extract also stimulated sexual reproduction in one strain of *Phytophthora drechsleri*, but not in *P. capsici*, *P. cambivora*, *P. citrophthora*, *P. cryptogea*, *P. palmivora*, or *P. parasitica*.

ABSTRACTS OF PAPERS SCHEDULED FOR THE JOINT MEETING OF THE POTATO ASSOCIATION OF AMERICA AND THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, DECEMBER 11, 1951

Verticillium wilt of potatoes. AYERS, G. W. Potato varieties differ widely in their susceptibility to Verticillium wilt. The seed potato inspection service in Prince Edward Island has rejected, for wilt, large acreages of Irish Cobbler and Sebago during the years that these varieties have been under inspection. Wilt is not common in Green Mountain and Katahdin and rarely have plantings of these varieties been rejected for this disease. The variety Houma possesses greater resistance to Verticillium wilt than any varieties now grown on a commercial scale. A high degree of immunity has been recorded in a scab-resistant seedling. Field experiments have demonstrated that in resistant varieties the organism does not readily become established as internal mycelium in tubers from plants grown in Verticillium-infested soil. Seed treatment with organic mercuries is an effective means of reducing field wilt through the elimination of all surface-borne inoculum. It is apparent that certain climatological or soil factors affect the occurrence of wilt. The disease has never become important in Kings County, Prince Edward Island. Seed of the Sebago variety when planted in the Southern United States has failed to develop wilt symptoms.

Mycorrhizalike mycelium in potato tubers. COOPER, D. C., G. H. RIEMAN, R. W. HOUGAS, G. W. STOKES, AND E. C. GASIORKIEWICZ. A mycorrhizalike mycelium is regularly associated with certain tissues of the potato tuber. An examination of 49 varieties and strains obtained from various regions in Wisconsin and of commercial varieties from eight other potato-producing States has revealed the presence of the fungus in the tuber in each instance. The fungus was likewise found to be present in the tubers of six tuber-bearing species of *Solanum* other than *S. tuberosum*. The fungus penetrates the apex of the stolon, passes through the cortical layers of cells, and becomes associated with the phloem parenchyma. Shortly thereafter tuberization is initiated. The nonseptate mycelium is both inter- and intra-cellular. An occasional host cell becomes filled with a coiled mycelium. Preliminary tests wherein plants were grown from true seed on a sterile substrate indicate that these mycorrhiza associations in the potato may be the cause of tuberization, since numerous stolons were produced without tuber formation.

Internal browning of potatoes as affected by date of planting and storage. ELLISON, J. HOWARD.

Factors influencing tuber discoloration following vine killing. HOYMAN, WM. G. Chemical vine killers were first used extensively in North Dakota in 1946, a year in which the rainfall was below normal. During the 1946 harvest, considerable vascular discoloration of the tubers was observed in some fields where vines had been killed. During the ensuing 5 years, Triumph potatoes were grown under conditions that provided different amounts of available soil moisture: 1) with natural rainfall, 2) with natural rainfall and irrigation, and 3) with natural rainfall but with the potatoes on elevated rows 2–4 ft. higher than the surrounding soil. Beginning in the forepart of August, the vines were destroyed at 10- to 14-day intervals by spraying with a chemical or cutting at the soil level. During this 5-year period, there was significantly more discoloration in tubers harvested from the elevated rows. Regardless of how grown, more discoloration tended to appear in tubers harvested from vines killed the forepart of August. The soil temperature in the region of tuber formation was approximately the same under all conditions and was not considered to be a factor influencing discoloration.

Internal browning of Netted Gem potatoes in California. KENDRICK, JAMES B., SR. Netted Gem potatoes grown in the Klamath Basin area of California frequently show two types of internal browning, net necrosis and stem-end browning. Net necrosis is definitely associated with current-season leaf-roll virus infection. Stem-end browning is not caused by any known virus infection but is definitely associated with length of growing season and soil type. The discoloration is darker brown than that of net necrosis, with a diffuse yellowing of the adjacent parenchyma. Stem-end browning rarely extends more than 1 in. deep. In 1947 and in 1950 the growing season extended well beyond the normal for this area because of the lateness of killing frosts, and in both years internal browning was severe. Net necrosis in 1947 was correlated with a high incidence of current-season leaf-roll infection, and stem-end browning occurred mainly in potatoes produced in muck soil. In 1950 internal browning was primarily of the stem-end type, penetrated deeper than in 1947, and was confined to potatoes grown on muck soil. Potatoes grown both years in sandy soil in the same area, and in some cases from the same seed lot, showed only slight to no stem-end browning.

Studies of dry fungicides on cut potato seed pieces. LANE, GEORGE H. Cut potato seed pieces treated with several organic and inorganic fungicides were planted in soil infested with Fusaria in the greenhouse. Thiram and zineb at low concentrations showed no detectable deleterious effect on the seed and were outstanding in reducing fungal attack. Field tests of these materials have been completed at two widely separated localities in Colorado in 1950 and 1951. Under adverse conditions of (a) storage of cut seed and (b) soil temperature and moisture at planting, each of these materials has reduced rot, improved stands, and increased yields significantly when compared to untreated cut seed. No significant differences were observed in the effectiveness of the two fungicides.

Mutations of potato virus X induced by nitrogen mustard. LARSON, R. H., M. A. STAHMANN, J. C. WALKER, AND R. W. COLBURN. A culture of the spot strain of potato virus X which has shown no apparent spontaneous mutation during numerous successive local lesion serial transfers over a number of years was used. Plants of *Nicotiana rustica* L. were exposed to vapor of methyl-bis (β-chloroethyl) amine *in vivo* shortly after inoculation. Exposure was made by introducing vapors of nitrogen mustard into a glass chamber containing the inoculated *N. rustica* plants until the vapor concentration was about 0.25 mgm. of the amine per liter. This exposure was repeated four times at 24-hr. intervals at about 20° C. The needle technique was used in transfers from chlorotic lesions as they appeared on inoculated leaves 5–7 days after treatment. Subsequent subtransfers to *N. rustica* of isolates originating from single lesions resulted in very marked differences in symptoms. The mutants were classified into four types. These types were transferred repeatedly to *N. rustica* without reversion to the original spot strain of virus X. The protective action of X mottle (X^H strain) on *Datura stramonium* L. was similar against the four mutant types and the spot strain. Exposure of the virus *in vitro* to a solution of the nitrogen mustard failed to produce mutants.

Inoculation of potatoes with ring rot tissue from different sources. LOGSDON, CHARLES E. When six varieties of potatoes were inoculated with ring rot tissue from tubers of each of the same six varieties, the average percentages of infection with all sources of inoculum were as follows: Triumph 49, Cobbler 46, Green Mountain 29, Red Warba 26, Sebago 24, and Teton 12. The average percentages of infection on all varieties with inoculum from Cobbler was 35, from Triumph 35, from Teton 34, from Sebago 32, from Green Mountain 25, and from Red Warba 23. Eight tenths of a gram of diseased tissue in 2 gal. water was used as inoculum as well as 1/3, 1/9, and 1/27 of the basic concentration. There was no significant reduction in the percentage infection resulting from the lower concentrations. Thus there are real differences in the virulence of the ring rot bacteria from different potato varieties which had been inoculated the previous year with the same mixture of bacteria. However, the most resistant varieties do not always yield the most virulent inoculum. There was a tendency for inoculum from some sources to reverse their virulence on different varieties, but these interactions were not statistically significant.

Results of testing lines and varieties of potatoes for field resistance to Verticillium wilt. McLEAN, JOHN G. Verticillium wilt of potatoes has been estimated to cause from 20 to 25 per cent loss in the crop of Russet Burbank potatoes in the upper Snake River Valley of Idaho. To determine sources of resistance to the disease, some 1000 lines and varieties have been tested at Aberdeen and at Rexburg, Idaho. A significant correlation of –0.836 was obtained between yield and index of Verticillium infection, with yields ranging from 554 sacks per acre for a resistant variety to 174 for a susceptible. A strong correlation was found between lateness of the variety and apparent resistance. The best sources of resistance were found in Katahdin and Seedling 41956 and their relatives, and in relatives of Jubel and other foreign varieties. The variety Populair has shown the greatest resistance to date; Cobbler, Kennebec, Russet Burbank and X 96-56 were very susceptible. Environmental conditions of heat or drought appeared to reduce the resistance in some varieties but not in others.

The development of races of Phytophthora infestans (Mont.) deBary on potato hybrids. MILLS, W. R., AND L. C. PETERSON. Three distinct genes for late blight immunity, designated as genes B, C, and D, have been identified among progeny of hybrids of *Solanum demissum* by *S. tuberosum*. Immunity is dominant over susceptibility. As the immune plants approach maturity, immunity gives way to a high degree of resistance. Serial passages of the common field race A through senescent foliage of such plants result in the development of new virulent races of the pathogen, highly stable and specific for the gene or genes carried by the host plant. Thus race B is formed by passage of race A through plants carrying gene B. Since eight different combinations of the three plant genes are possible, eight races of *P. infestans* were postulated, including race A which is virulent for the completely recessive form only. Five of the seven expected races have been repeatedly developed in the laboratory, and isolated from breeding plots. All attempts to develop the remaining two races (CD and BCD) or to isolate them from the field have failed. Plants of genetic constitutions CD and BCD have remained blight-free. Failure to find or produce races combining virulence factors C and D suggests that these factors are allelomorphic.

Effects of seed-piece treatment on yields and seed-piece decay of potato. OLMSTED, DONALD S. In 1950 dipping cut or whole Cobbler potato seed in a 5 per cent Zerlate suspension increased yields about 100 per cent. Phygon, Spergon, and Arasan also increased yields appreciably. In 1951 cut seed of Red Warba, Cobbler, and Pontiac were dipped in several concentrations of Zerlate, Zinc oxide, Phygon, and Semesan Bel. Two identical experiments were planted 2 weeks apart. Zerlate, 7 per cent, Zinc oxide, 3 per cent, and Phygon, 0.5 per cent resulted in consistent yield increases of 13 to 43 per cent on early plantings of all three varieties. All treatments of early plantings of Warba and Pontiac exceeded the checks in yield and all treatments of late plantings of these varieties yielded less than the checks, except 3 per cent Zerlate on Warba. Yields from treated Cobbler all exceeded the checks, regardless of planting date. No disease except seed-piece decay was apparent. Seed-piece decay was not associated with yield, except that, in general, less decay occurred in early plantings of Warba and Pontiac, whereas the reverse was true of Cobbler.

Fungicidal treatment of seed potatoes after cutting. RICH, AVERY E. Sixteen different chemicals were used as instant dips, and seven were used as dusts, in an attempt to control Fusarium seed-piece decay in the field and in the greenhouse. CC 224 (a mercury zinc chromate) and Semesan Bel reduced the stand in the field in the absence of the disease. Agrox (phenyl mercury urea) treatments resulted in a high percentage of seed-piece decay in the greenhouse. Dusting the cut seed pieces with Phygon, Spergon, Parzate, Zerlate, Arasan, or Ortho 406 gave satisfactory control even in contaminated greenhouse soil. Excessive amounts of Spergon were injurious, however. The fungicides included in the 1951 field trials were given a severe test. The cut seed pieces were inoculated before treatment by dipping them in a slurry of rotted potatoes and water. Under these severe conditions Parzate, Zerlate, and Vancide 51 were ineffective in improving stands. Semesan Bel, Phygon, Ortho 406, and Manganese ethylene bis-dithiocarbamate treatments produced almost perfect stands, as compared to 82 per cent for the inoculated but untreated seed pieces.

Persistence of scab resistance in potato varieties. RIEMAN, G. H., R. W. HOUGAS, AND G. STOKES. Hindenburg and Irish Cobbler seed pieces infected with common scab of potatoes, caused by *Streptomyces scabies* (Thaxt.) Waksman & Henrici, were planted in isolated field plots for five consecutive years. Increased amounts of scab infection developed on the tubers of the susceptible variety Irish Cobbler, whereas only trace amounts were produced on the tubers of the more or less resistant variety Hindenburg. Disease development was more severe in amount and type on Irish Cobbler following repeated plantings of the same variety than when it was grown following like plantings of the resistant variety. These results suggest that different physiologic races of the pathogen parasitize resistant and susceptible potato varieties in the field. No differences were observed in the incidence of scab on the resistant variety following plantings of either resistant or susceptible potatoes. Experimental evidence has been secured indicating that scab resistance in potatoes in the field tends to remain constant over several years.

Immunological and histological relationship between strains of the potato leaf-roll virus. WEBB, R. E., AND R. H. LARSON. Four leaf-roll strains were found to immunize the host, *Physalis floridana* Rydb., against one another after the onset of macroscopic symptoms. All attempts to recover the challenge strain of the virus failed after a 7-, 14-, or 21-day incubation period in the host. In all cases only the immunizing strain was recovered from the inoculated host. Histological examination of stems of *P. floridana* infected with each of the four strains of the leaf-roll virus showed a high degree of correlation between the extent of phloem necrosis and severity of external symptoms. The mild strain, No. 1, caused slight phloem necrosis only in isolated inner phloem groups with very few cells of a group affected. Cambial activity was slightly retarded in comparison to the control. Strain 2 caused somewhat more phloem necrosis and less cambial activity than strain 1. Severe external and internal phloem necrosis and severe disorientation of the stem tissues with greatly reduced and irregular cambial activity resulted from infection with strain 3. With the severe strain, No. 4, almost complete destruction of the external and internal primary phloem greatly reduced cambial activity, and internal modification of the stem tissues resulted in death of the plants.

Control of potato seed-piece decay by seed-piece treatment. YOUNG, ROY A., AND J. A. MILBRATH. During the past 3 years trials have been conducted to determine the effectiveness of fungicidal treatments of cut potato seed-pieces in preventing seed-piece decay caused by *Fusarium* and *Pythium* spp. Observations have been made on seed-piece decay, disease incidence, stand, and yield. Treatment with Semesan Bel, Phygon, and ziram resulted in reduced seed-piece decay, a reduction in the incidence of blackleg and rhizoctonia, and increased stand and yield. Of particular interest were the results obtained using a tuber unit potato planter adapted for treatment of cut potato seed-pieces. The feature of cutting, treating, and planting in one operation facilitates the use of mercurial fungicides which frequently are injurious if seed-pieces are not planted shortly after treatment. In 1951, treatments with Phygon, Semesan Bel, and ziram resulted in stand increases up to 26 per cent and yield increases of up to 2500 lb. per acre. There was also a marked reduction in the incidence of blackleg and rhizoctonia in some plots. In a trial on peat soil, seed lots treated with Semesan Bel averaged 34, Phygon 127, ziram 159, water 247, and untreated 196 diseased plants in 4800.

TRANSMISSION OF ALFALFA WITCH'S BROOM TO NONLEGUMINOUS PLANTS BY DODDER, AND CURE IN PERIWINKLE BY HEAT[1]

L. O. Kunkel

In a recent paper Klostermeyer and Menzies (5) report transmission of alfalfa witch's broom to 15 species in seven genera of leguminous plants by the leafhopper *Scaphytopius (Cloanthanus) dubius* (Van Duzee). Only one species, the potato, outside of the Leguminosae was tested. Potato plants exposed to infective leafhoppers for a period of 13 days did not take the disease. Whether this failure to transmit was due to immunity in the potato or to some other cause was not shown.

In work on alfalfa witch's broom, obtained from Dr. J. D. Menzies through the kindness of Dr. L. M. Black in 1942, the writer transmitted the virus causing this disease to four nonleguminous species including the potato and the periwinkle. The chief object of the work was to determine whether the alfalfa disease in periwinkles could be cured by heat, and to compare symptoms caused by this disease with those caused by the potato witch's broom in mutual suscepts.[2] The work also included experiments made in unsuccessful attempts to transmit alfalfa witch's-broom virus to alfalfa and parsley plants by dodder and to carrot and aster plants by the aster leafhopper *Macrosteles divisus* Uhler. This paper records the results obtained.

MATERIALS AND METHODS.—Virus-free dodder, grown from seeds of *Cuscuta campestris* Yuncker and cultured routinely on healthy sugar-beet plants, was employed in all dodder-transmission experiments. The leafhoppers used were taken from a stock that had been maintained on healthy rye plants in insect cages for many years. All periwinkle plants were grown from cuttings belonging to a single clone. Potato plants were grown from tubers of the variety Jersey Red Skin. Alfalfa, carrot, tomato, and parsley plants were grown from seeds purchased from commercial seed houses. The carrots were of the variety Tendersweet, and the tomatoes of the variety Bonny Best. All plants except the potatoes were believed to be virus-free when taken for use in experiments. The potatoes were infected by a mild strain of the X-virus of potato. Plants were grown in rich garden soil in porous clay pots, kept in greenhouses. The methods employed in dodder and in insect-transmission experiments and in heat-treatment experiments were similar to those described in previous publications (1, 2, 3, 4). All grafts were of the cleft type.

DODDER TRANSMISSION EXPERIMENTS.—One of several experiments in which alfalfa witch's broom was transmitted to carrot plants by dodder will be described in detail. Dodder that had been growing on a witch's-broom alfalfa plant for about 6 months was allowed to parasitize each of 18 healthy young carrot plants for 30 days. Dodder that had been growing on a healthy alfalfa plant for the same length of time was allowed to parasitize 18 other healthy young carrot plants for the same number of days. All dodder was then removed and destroyed. Thirty-eight days after this was done, two of the carrot plants exposed to virus-bearing dodder showed early symptoms of witch's broom. During the next 105 days, at the end of which time the experiment was terminated, 10 more of the carrots exposed to virus-carrying dodder became diseased. The other six plants and the 18 control plants were still healthy in appearance. In other similar experiments with carrots, witch's broom was transmitted to three of ten plants, to two of two plants, to four of six plants, and to 16 of 16 plants exposed to virus-carrying dodder for 30 days or less. Equal numbers of control plants exposed to virus-free dodder remained healthy. In these experiments plants were held under observation for about 3 months after the dodder was removed. The shortest period between the time virus-carrying dodder was placed on carrot plants and the first appearance of symptoms was 43 days.

In still other similar experiments with periwinkle plants, alfalfa witch's-broom virus was transmitted to two of two and to five of six plants exposed to virus-carrying dodder for about one month. Equal numbers of control plants exposed to virus-free dodder for the same length of time remained healthy in appearance. The first symptoms of witch's broom appeared in one of the plants of *Vinca rosea* 97 days after it was first exposed; all of the other periwinkle plants required a somewhat longer incubation period.

In three further similar experiments, in which tomato plants were exposed to witch's-broom virus by means of dodder, the disease was taken to one of five plants, to four of five plants, and to one of six plants used in the experiments. Early symptoms appeared in these plants within about 3 months after they were first exposed. Equal numbers of control plants, exposed to virus-free dodder, remained healthy. All plants were held under observation for 5 months after they were first exposed.

A number of similar dodder-transmission experiments with alfalfa plants were carried out over a period of 34 months beginning soon after diseased plants were first received on June 2, 1942. In the earliest experiment, virus-free dodder was allowed to grow on a diseased alfalfa plant for 32 days. Branches of dodder that had developed on this plant were removed and placed on six healthy young alfalfa plants. At the same time, virus-free dodder that had developed on a healthy alfalfa plant was placed on six other healthy young alfalfa plants. After the dodder had parasitized the plants for about 6 months, it was removed and de-

[1] Accepted for publication July 28, 1951.

[2] The virus causing potato witch's broom was originally obtained from Dr. Donald Folsom of the Maine Agricultural Experiment Station in 1940.

stroyed. These dodder-free plants were kept under observation for 233 days. None showed symptoms of disease during this time.

When it became evident that it would not be easy to transmit alfalfa witch's-broom virus from alfalfa to alfalfa by means of dodder, larger experiments were made. One of these will be described in detail. Dodder that had been growing on a diseased alfalfa plant for 6 months was placed on each of 40 very young, healthy alfalfa seedlings. At the same time, virus-free dodder that had been growing on a healthy alfalfa plant for more than 6 months was placed on eight other very young, healthy alfalfa seedlings. The dodder remained on the plants for 151 days before it was removed and destroyed. During this period it was pruned back from time to time to prevent its killing the plants. Nevertheless, two of the 40 plants on which virus-bearing dodder was growing died, probably because they were damaged too severely by the parasite. The other 38 plants exposed to virus-bearing dodder and the eight control plants exposed to virus-free dodder were kept under observation for more than one year after the dodder was removed. At no time did any of them show symptoms of disease. In spite of persistent efforts made during a period of almost three years, all attempts to transmit alfalfa witch's-broom virus from diseased plants of alfalfa, carrot, and *Vinca rosea* to alfalfa by dodder failed.

FIG. 1. A carrot plant to which alfalfa witch's-broom virus was transmitted by dodder, showing an abundant production of flowers. (Photograph by J. A. Carlile.)

In a single experiment an effort was made to transmit alfalfa witch's-broom virus from alfalfa to parsley plants. Dodder that had been growing on a diseased alfalfa plant for more than a month was placed on each of 12 healthy young parsley plants. At the same time, virus-free dodder that had been growing for more than a month on a healthy alfalfa plant was placed on each of four healthy young parsley plants. After the dodder had parasitized the plants for 2 months it was removed and destroyed. The plants were kept under observation for a little more than 5 months following this operation. All of the plants grew well and became large. None showed disease.

TESTS OF THE ABILITY OF THE ASTER LEAFHOPPER MACROSTELES DIVISUS TO TRANSMIT ALFALFA WITCH'S-BROOM VIRUS.—It was not expected that the aster leafhopper would transmit alfalfa witch's-broom virus but, since the virus readily infects carrots, on which the aster leafhopper thrives and to which it transmits aster yellows, the possibility that it might transmit this virus was tested. A colony of aster leafhoppers that had fed on a witch's-broom carrot plant for 2 weeks was allowed to feed for from 1 to 3 days on each plant in a succession of 16 aster and 17 carrot plants placed alternately in a series. The insects were kept on the test plants for a total period of 41 days. At the beginning of the test the colony consisted of 50 individuals. By the end of the 41-day period it had dwindled to 12. The plants were kept under observation for 3 months following their exposure. None became diseased. Three other similar experiments with carrot and aster plants gave negative results.

TRANSMISSIONS BY GRAFTING.—Alfalfa witch's-broom virus was maintained in alfalfa and tomato plants by means of a series of graft transmissions carried out over a period of 2 years. The virus was first taken to potato plants by means of diseased tomato scions. It was maintained in potato plants for 3 years by a series of grafts in which potato scions were used. The virus was taken to many periwinkle plants by grafting. It has been maintained in this species by semiannual graft transmissions during the past 9 years. It is still being maintained in this way, as is also the potato witch's-broom virus.

SYMPTOMS ON NONLEGUMINOUS PLANTS.—On carrots the alfalfa witch's-broom virus produced symptoms similar to but somewhat less severe than those of aster yellows. It caused lengthening and distortion of leaf petioles and a general chlorosis. Also, it stimulated the development of secondary shoots. It did not depress flowering to the same extent as does aster yellows. Figure 1 shows a carrot plant that had been diseased for several months. Most of the secondary shoots bore normal-appearing flowers.

In tomato the disease caused mild chlorosis and some stimulation of secondary shoots. It was not observed to cause malformations or virescence in flowers. Plants that had been diseased for some time continued to bear normal yellow flowers and undersized but otherwise normal-appearing fruits.

Alfalfa witch's broom in potato caused stunting, a mild type of chlorosis, and considerable but not excessive stimulation of secondary shoots. The degree of chlorosis produced is illustrated in figure 2 where a leaf from a healthy and one from a diseased plant are shown. Alfalfa witch's broom in potato caused the production of a rather coarse witch's-broom type of growth (Fig. 3), in contrast to the thin wiry type of growth characteristic of the witch's broom of potato from Maine (Fig. 4). It also differed from the potato witch's-broom disease of the West described by Young and Morris (8). Alfalfa witch's-broom virus moved readily in potato plants and greatly reduced the sizes and numbers of tubers.

The first symptom of alfalfa witch's broom in large periwinkle plants was a gradual fading of the pink color of flowers. Somewhat later the flowers were almost white and were considerably reduced in size. Instead of falling off as do corollas of normal flowers, the corollas of diseased flowers dried out and remained attached. Frequently a bud developed in the base of the style. This produced either another abnormal flower or a vegetative shoot. As the disease progressed, some of the corollas became green and did not dry out and die. Finally, no more flowers were produced. The foliage was very chlorotic and many thin secondary shoots developed to cause a witch's-broom type of growth, such as is shown in figure 5.

CURE OF ALFALFA WITCH'S BROOM IN PERIWINKLE BY HEAT. — Diseased periwinkle plants were given heat treatments at 30°, 32.5°, 35°, 40°, and 42° C. for different periods of time. Seven plants were treated for periods varying by 5-day intervals for from 5 to 35 days at 30° C. None were cured and none showed any marked beneficial effects from the treatments. Six plants were treated for periods varying by 5-day intervals for from 5 to 30 days at 32.5° C. The plants held at this temperature for 25 and 30 days were cured. The four plants held at 32.5° C. for 20 days or less were not cured. Five plants were treated at 35° C. for periods varying by 5-day intervals for from 5 to 25 days. The plant treated for 25 days at this tem-

FIG. 3. A plant of the potato variety Jersey Red Skin, showing the witch's-broom type of growth caused by alfalfa witch's-broom virus. (Photograph by J. A. Carlile.)

FIG. 2. Leaves from potato plants of the variety Jersey Red Skin; the leaf at the left was from a healthy plant, that at the right from a diseased plant that showed the typical chlorosis caused by alfalfa witch's broom. (Photograph by J. A. Carlile.)

FIG. 4. A seedling potato plant affected by potato witch's broom from Maine. (Photograph by J. A. Carlile.)

FIG. 5. Two periwinkle plants of the same age that were equally affected by alfalfa witch's broom when the plant at the right was given a 10-day heat treatment at 42° C. The picture was taken 3½ months after treatment. The treated plant was cured. (Photograph by J. A. Carlile.)

perature was cured: that treated for 20 days appeared to be cured for about 2 months but later produced diseased branches from its base. Plants treated at 35° C. for 20 days or less were not cured. Four plants were treated at 40° C. for periods varying by 5-day intervals for from 5 to 20 days. The three plants held at this temperature for 10, 15, and 20 days, respectively, were cured. The plant held at 40° C. for 5 days was not cured. Six plants were treated at 42° C. for periods varying by one-day intervals for from 5 to 10 days. The plants treated at this temperature for 5 and 6 days were not cured; those treated for 7, 8, 9, and 10 days, respectively, were cured. Figure 5 shows two plants of the same age that were equally affected by alfalfa witch's broom when the plant at the right was given a 10-day heat treatment at 42° C. The picture was taken 105 days after the treatment was ended. As may be seen, the treated plant was healthy and was producing normal blossoms while the untreated plant was badly diseased. Although the plants treated at 42° C. for 5 and 6 days were not cured, they showed beneficial effects from the treatments. Most of the branches produced normal-appearing leaves for several weeks after treatment. However, diseased branches developed from the main stems of these plants. After a few months, all branches were diseased.

After the two periwinkle plants that had been cured by exposure at 42° C. for 8 and 9 days, respectively, had been held under observation for a year and 4 months, both were inoculated by grafting diseased periwinkle scions into their tops. In due course, both came down with the alfalfa witch's-broom disease, thus proving that cured periwinkles were not immune from the disease.

DISCUSSION.—The identity of the virus transmitted from alfalfa to nonleguminous plants by dodder might be questioned since the virus could not be taken from nonleguminous plants to alfalfa and shown to produce the symptoms of alfalfa witch's broom in the latter. However, the symptoms in nonleguminous hosts were sufficiently like those in alfalfa to minimize doubts on this point. Also, it seemed unlikely that two or more viruses could have been present in the plants received from Dr. Menzies since mechanically transmissible viruses were absent, and any present in the plants must have been transmitted to them by the vector of

alfalfa witch's broom. Hence, it was assumed that the virus transmitted from alfalfa to nonleguminous plants was that of alfalfa witch's broom.

This report of work on alfalfa witch's broom furnishes another demonstration of the great value of dodder as a transmitting agent for viruses in the yellows group. Transmission of alfalfa witch's-broom virus to potato proved that failure of the insect vector (5) to transmit to potato was not due to immunity in this plant. By taking alfalfa witch's-broom virus to tomato, potato, and periwinkle plants, it was possible to compare the symptoms it produced in these plants with those caused by potato witch's-broom virus and by a number of other viruses of yellows type diseases in the same hosts. The comparison led to the conclusion that alfalfa witch's broom was distinct from potato witch's broom and, in fact, from all other yellows type diseases that have been taken to any or all of these hosts. The writer confirmed Menzies' (6, 7) finding that alfalfa witch's broom was not transmitted to alfalfa by the dodder species used. No explanation can be offered at present for this failure. Alfalfa, being a natural host of the virus, was apparently very susceptible. The dodder grew well on alfalfa and there was no apparent reason why it did not transmit to this plant just as it did to carrot, tomato, and periwinkle plants. However, it should be pointed out that this is not the only instance in which dodder has shown an anomalous behavior in respect to transmission of viruses. Peach rosette and peach X-disease virus are readily transmitted from peach to carrot, tomato, and periwinkle but not from peach, carrot, tomato, or periwinkle to peach, the natural host of these viruses. The aster yellows virus is readily transmitted to red clover by the dodder *Cuscuta campestris*, but is transmitted with difficulty to China aster, the preferred host of this virus. On the other hand, it is transmitted to China aster very readily by the aster leafhopper but rarely and with difficulty to red clover by this vector. If only dodder transmission experiments were made, it would be concluded that red clover is susceptible but the China aster resistant; whereas, if only insect transmission experiments were carried out, it would be concluded that the China aster is highly susceptible but red clover very resistant.

Alfalfa witch's broom in periwinkles was readily cured by heat treatments at temperatures near 35° C. At this temperature it showed a sensitivity in periwinkles somewhat comparable to that shown by peach yellows in peach seedlings (2), but at 42° C. it was far more resistant than the peach yellows virus. It was considerably more sensitive to heat than the potato witch's-broom virus (4).

SUMMARY

Alfalfa witch's-broom virus was transmitted from alfalfa to carrot, tomato, and periwinkle but not to alfalfa or parsley by the dodder *Cuscuta campestris*.

It was transmitted from alfalfa to alfalfa, from tomato to tomato, from tomato to potato, from potato to potato, and from periwinkle to periwinkle by grafting.

It was not transmitted from diseased to healthy carrots or from diseased carrots to healthy China aster plants by the aster leafhopper *Macrosteles divisus*.

A comparison of symptoms in tomato, potato, and periwinkle plants with symptoms of potato witch's broom in the same plants indicated that the two diseases were quite distinct. Symptoms in these and in carrot plants indicated that alfalfa witch's broom was not like any of the other well known yellows type diseases.

Alfalfa witch's broom was not cured in periwinkles by treatments at 30° C. for as long as 35 days. It was cured by treatments at 32.5° C. and at 35° C. lasting 25 but not by treatments lasting 20 days; by a treatment at 40° C. lasting 10 but not by a treatment lasting 5 days; and by a treatment at 42° C. lasting 7 but not by a treatment lasting 6 days. Cured plants were susceptible to alfalfa witch's-broom disease.

THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, NEW YORK

LITERATURE CITED

1. KUNKEL, L. O. Studies on aster yellows. Amer. Jour. Bot. **13**:646-705. 1926.
2. ———. Heat treatments for the cure of yellows and other virus diseases of peach. Phytopath. **26**:809-830. 1936.
3. ———. Heat cure of aster yellows in periwinkles. Amer. Jour. Bot. **28**:761-769. 1941.
4. ———. Potato witch's broom transmission by dodder and cure by heat. Proc. Amer. Philos. Soc. **86**:470-475. 1943.
5. KLOSTERMEYER, E. C., AND J. D. MENZIES. Insect transmission of alfalfa witch's-broom virus to other legumes. Phytopath. **41**:456-458. 1951.
6. MENZIES, J. D. Transmission studies with alfalfa witches' broom. (Abstr.) Phytopath. **34**:936. 1944.
7. ———. Witches' broom of alfalfa in North America. Phytopath. **36**:762-774. 1946.
8. YOUNG, P. A., AND H. E. MORRIS. Witches' broom of potatoes and tomatoes. Jour. Agr. Res. [U. S.] **36**:835-854. 1928.

PHYTOPHTHORA ROOT ROT OF SAFFLOWER[1]

Donald C. Erwin[2]

In 1947 root rot of safflower (*Carthamus tinctorius* L.) was first observed in western Nebraska. In 1949 Claassen et al. (2) reported that the disease occurred on plants of susceptible varieties at nearly all stages of growth, and that it was most prevalent on irrigated land. In one instance 80 per cent of all the plants in one field were killed by root rot. Fortunately losses may be diminished by the use of resistant varieties (3); however, some of the varieties yielding the highest percentage of oil from seed were highly susceptible to the root rot.

The causal agent of the disease has previously been reported by the writer (4) to be *Phytophthora drechsleri* Tucker.[3] The fungus caused root rot on safflower plants at all stages of growth from pre-emergence to near maturity. On succulent plants 2 to 3 weeks old the first visible symptoms were water-soaking and collapse of cortical tissues of the lower stems. The plants then fell over and wilted. On older plants near the blossom stage, black necrotic lesions encompassed the roots and sometimes extended 1 or 2 in. above the ground on the lower part of the stems; leaves sometimes turned yellow. The entire plant then wilted and many plants died 5 to 10 days later. The cortex of diseased roots ranged in color from dark brown to greenish-black. In advanced stages the vascular tissues and pith also became necrotic and dark-colored.

Since the disease has caused serious crop losses in Nebraska when susceptible varieties were used, an investigation of the effects of some environmental factors on the disease and on the pathogen seemed desirable. *Phytophthora drechsleri* has been adequately described (8, 9); therefore, no morphological description of the fungus will be given.

PATHOGENICITY TESTS.—Isolations were made from safflower plants, grown in the Scottsbluff area, that had typical symptoms of root rot. Each isolate obtained was tested on safflower plants growing in 5- or 7-in. pots: the inoculum was placed in a shallow trench in the soil adjacent to the plants, after some lateral roots were wounded by passing a knife through the soil about 1 in. from the plants. Except where otherwise designated, this method was used throughout the study. Inserting a piece of mycelium into the lower stem was also an effective method of inoculation. Adding inoculum to the lower half of the soil in 5-in. pots caused 100 per cent pre-emergent seedling loss. Because of the severe pathogenicity of the fungus on germinating seedlings, this method was not used further.

Safflower plants of the variety N-9 were grown from seeds which were sterilized by soaking 3 min. in a 1:1000 solution of mercuric chloride followed by washing in distilled water. No deleterious effects of the mercuric chloride on germination of the seed were detected.

The inoculum used in tests to prove the pathogenicity of *P. drechsleri* consisted of four plate cultures, incubated 1 week on potato-dextrose agar and macerated in 1000 ml. of distilled water for 10 sec. in a Waring Blendor.

In all of nine separate pathogenicity tests, more than 50 per cent of the plants were killed. A high incidence of disease was obtained on young and old plants growing in nonsterilized as well as sterilized soil; however, the greater percentage of root rot occurred in sterilized soil. Recent tests show that infection may occur in nonwounded roots.

P. drechsleri was not pathogenic on stems or roots of tobacco, pepper, potato, or Great Northern bean plants. It has been reported to cause a storage rot of potato tubers at high temperatures (5) in Nebraska; however, it has not previously been reported to be pathogenic on growing plants of any crop other than safflower in Nebraska.

EFFECT OF TEMPERATURE ON GROWTH OF THE PATHOGEN.—Since growth in culture at different temperatures is an important criterion in the identification of *Phytophthora* species (9), experiments were made to determine the cardinal temperatures for growth of the organism on cornmeal, malt extract, and potato-dextrose agars. Five to eight plates were each inoculated with a 4-mm. disk of mycelium from the periphery of a 4-day-old culture and incubated for 72 hr. The radial growth was determined by averaging two diameters of each colony.

A profuse amount of aerial mycelium occurred on potato-dextrose and malt extract agars, whereas on cornmeal agar the mycelium was appressed. Although the total radial growth varied from one test to another, the greatest amount in each test occurred at 30° C. (Fig. 1). The average diameters of colonies in the range of 32.5° to 37.5° C. was fairly high, but close inspection of the colonies revealed that the mycelium was much more sparse at the higher temperatures. Only slight growth occurred in two of five plates at 40° C. Results of this experiment are in close agreement to those of Tucker (9) and Tompkins et al. (8).

[1] Accepted for publication July 30, 1951.

Portion of a thesis submitted in partial fulfillment of the requirements for the Master of Arts degree, University of Nebraska. Published with the approval of the Director as Paper 515, Journal Series, Nebraska Agricultural Experiment Station.

This study was made possible by funds provided by the Nathan J. Gold Research Fellowship.

[2] Graduate Assistant, Division of Plant Pathology, University of California, Davis, California. The author acknowledges the guidance of Dr. J. E. Livingston of the Department of Plant Pathology of the University of Nebraska, under whose supervision the work was done.

[3] The author wishes to express appreciation to Dr. C. M. Tucker for identification of the organism.

EFFECT OF HYDROGEN-ION CONCENTRATION ON GROWTH OF THE PATHOGEN.—*P. drechsleri* was grown on cornmeal agar adjusted to pH values of 4.0, 5.0, 6.0, 7.0, and 8.0 with N/20 NaOH or N/20 HCl after sterilization. Values were determined on a Beckman pH meter. The inoculation method was the same as that used in the temperature experiment. The average diameter of each colony was determined after incubation at 30° C. for 72 hr. Growth occurred at all pH values; the greatest amount at pH 7.0, with slightly less at 6.0 and 8.0. Radial growth at pH 4.0 and 5.0 was considerably less, the least occurring at pH 4.0. These data are not in agreement with those of Tompkins et al. (8), who reported good growth throughout a wide range of pH values.

After the agar was melted and the mycelium was separated from it, the pH was again determined. The changes were as follows: pH 4.0 to 4.2; 5.0 to 5.2; 6.0 to 5.9; 7.0 to 6.2; 8.0 to 6.5. The higher pH values of the medium were lowered by the growth of the fungus; at lower pH there was little change.

EFFECT OF SOIL TEMPERATURE ON DEVELOPMENT OF ROOT ROT.—An experiment was made and repeated once to determine the most favorable temperature range for root rot on young safflower plants. Soil was steam-sterilized for 3 hr. at 20 lb. pressure to decrease possible variation due to other soil organisms. In water-bath chambers controlled within 1° C. above or below the desired point (7), the soil temperature was maintained at 21° C. after planting until one week prior to inoculation to obtain plants of uniform size; thereafter, 17°, 20°, 25°, 30°, and 35° C. were maintained, using one chamber for each temperature. In both experiments the soil moisture was maintained at a point slightly above the moisture equivalent of the soil. The inoculum for the following experiments was grown for one week in 500 ml. of potato-dextrose liquid medium in 1000-ml. Erlenmeyer flasks, and the mycelium from one flask was then macerated in 1000 ml. of distilled water. This method largely eliminated the nutritive or staling effects that might be associated with a suspension of the organism in ordinary media.

Approximately 10 plants per can were grown in each of the eight soil cans in each chamber. The plants in two of the eight cans were used as controls, and those in the remaining six cans were inoculated with 200 ml. of macerated mycelium. In the first experiment the plants were inoculated on the 23rd day after planting and in the second experiment on the 21st day.

Soil temperature of 30° was slightly inhibitive for growth of safflower, at 35° C. growth was definitely retarded, and the most favorable range appeared to be 17°–25° C.

Data were compiled by removing plants showing symptoms of root rot every two days and recording the number diseased. In the first experiment the temperature most favorable for root rot was 25°, and in the second experiment, 30° C. (Fig. 2). Considerable root rot occurred at 35° C.; however, it was sometimes difficult to distinguish between heat-damaged and diseased plants. A 33 per cent loss in experiment 1 and

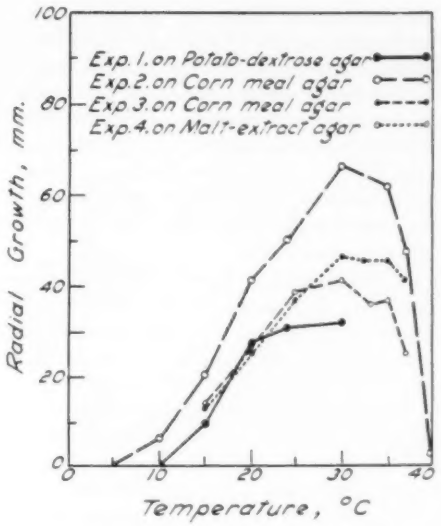

FIG. 1. The effect of temperature on radial growth of *Phytophthora drechsleri* in culture after 72 hr.

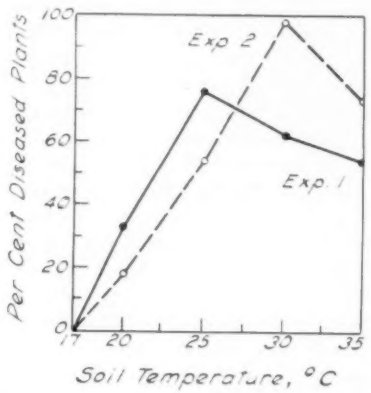

FIG. 2. The effect of soil temperature on the development of root rot of safflower 24 days after inoculation.

17 per cent in experiment 2 occurred in the 20° C. soil. At 17° C. no root rot occurred and all roots were healthy and free of lesions. Braun (1) reported that soil temperature below 15.5° C. was unfavorable for the development of root rot of guayule caused by *P. drechsleri*.

To determine whether the lack of disease at lower temperatures might be due to the failure of the inoculum to survive in the soil, two cans from the 17° chamber and two cans from the 20° chamber, in which none of the plants had symptoms of root rot, were placed in a greenhouse in which the air temperature was 27° C. In two weeks the plants in all four cans had symptoms of root rot. This indicated that the pathogen was viable, but relatively inactive, at the lower temperatures.

EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF ROOT ROT.—Since most of the root rot in Nebraska occurred on irrigated land (3), it seemed desirable to study the effect of high, medium, and low soil moisture on the percentage of plants killed or diseased.

The temperature of the soil was controlled at 25° except during the latter part of the experiment; then solar radiation caused soil temperature to rise as high as 30° C. for short periods. This did not appear to be a serious hindrance to the test. The soil used was a dark, silty clay-loam, three parts of which were mixed with one part of sand. The plants were grown for the first two weeks in cans, all of which were identical in weight, containing soil at 16.3 per cent moisture (moisture equivalent).

After the plants had emerged, a layer of coarse sand was spread over the surface of the soil in each can to diminish evaporation and the cans were divided into three lots. The moisture content of the medium-moisture soil was maintained by adding enough water to keep the soil at a point slightly higher than the moisture equivalent. A greater amount of water was added daily to those cans of soil being kept at high moisture. Each was kept at a nearly constant weight high enough to maintain the soil considerably above the moisture equivalent. The low-moisture group was maintained by adding only sufficient amounts of water each day to keep the plants from wilting permanently.

The soil moisture in the low-moisture lot could not be controlled at a moisture percentage less than the moisture equivalent, because watering dry soil to a point less than the moisture equivalent would result in the top layer's being moistened to the moisture equivalent, whereas the soil at the bottom would remain dry (6). However, it seemed that watering the plants in the low-moisture soil with small amounts probably simulated dry-land field conditions.

The error introduced by the weight increase of the growing plants was ignored since it was only a small fraction of a per cent of that of the entire soil mass.

The plants were inoculated in the usual manner. The diseased plants were removed every two days and the number recorded. At the termination of the test the roots of all apparently healthy plants were examined and the number of healthy and diseased roots in each lot was recorded (Table 1). At high and medium soil moisture nearly 100 per cent of the plants were diseased, whereas in low-moisture conditions only 57.6 per cent were diseased.

PATHOGENICITY OF THE FUNGUS ON PLANTS IN SAND AND NUTRIENT SOLUTION VS. SOIL.—This experiment was made and repeated once, to compare 1) the effect of two substrates on the development of root rot, and 2) the percentage of root rot occurring on sterilized with that on nonsterilized lots.

Each mixture was divided into a sterilized and a nonsterilized lot, and approximately 50 plants in each lot were inoculated. Approximately 25 safflower plants were grown as checks in each lot. The substrates consisted of 1) a dark, silty clay-loam soil, and 2) sand, which was irrigated daily with an excess of a modified Hoagland's solution[1] and flushed with water every third day to remove excess salts. The pH of each lot of soil and sand and nutrient solution was approximately 6.0.

When the plants were 22 days old, they were inoculated by pouring 100 ml. of inoculum into a trench near the plants. The plants which developed root rot were removed and the number recorded every two days. Since a high percentage of disease usually occurred on both substrates within a long incubation period, the data were compiled when 90 per cent or more of the plants grown on one lot were killed by root rot. Readings in the first experiment were made 17 days, and in the second experiment 15 days, after inoculation.

In both soil and sand less root rot occurred in the nonsterilized lots than in the sterilized. Ninety-three per cent of the plants in sterilized as compared to 82 per cent in nonsterilized sand were killed by root rot. Much less root rot occurred in the silty clay-loam soil than in the sand. In sterilized soil 22 per cent loss occurred; in nonsterilized soil 17 per cent of the plants were killed. It is possible that the greater percentage of disease in sand could be due to increased succulence of the host, since the plant growth was much more luxuriant in nutrient solution.

DISCUSSION.—Soil temperature is an important factor influencing the pathogenicity of *Phytophthora drechsleri* to safflower. The optimum temperatures for disease development (25° and 30°) correlated closely with the most favorable temperature for radial growth

[1] Composed of the following salts in molar concentration: $Ca(NO_3)_2.4H_2O$, 50 ml.; KNO_3, 50 ml.; NaCl, 22.2 ml.; $MgSO_4.7H_2O$, 20 ml.; KH_2PO_4, 10 ml.; 10 ml. of a solution consisting of 11 gm. of H_3BO_3 and 7 gm. $MnCl_2.4H_2O$ in 18 l. of water; H_2O, 10 l.

TABLE 1.—*The effect of high, medium, and low soil moisture on the development of root rot of safflower after inoculation with Phytophthora drechsleri*

Soil moisture	Treatment	Number of plants	Number of plants killed 24 days after inoculation	Number and root condition of apparently healthy plants			Percentage plants diseased
				Healthy	Brown	Necrotic	
High	Inoculated	109	89	3	9	8	97.2
	Check	30	0	30	0	0	0.0
Medium	Inoculated	109	78	17	3	11	84.4
	Check	23	3[a]	0	0	0	13.0[a]
Low	Inoculated	78	33	33	5	7	57.6
	Check	28	0	28	0	0	0.0

[a] The three check plants in the medium-moisture soil were killed by a dieback-type disease, the cause of which was not determined.

of the pathogen in culture (30° C.). Soil temperature of 17° was unfavorable for the development of root rot, and in all four tests the radial growth of the pathogen in culture was extremely slow at 15° C. Braun (1) found that root rot of guayule caused by *P. drechsleri* could be controlled by limiting irrigation to times when the soil temperature was below 18.3° C. No investigation of the feasibility of such a practice has been made with safflower. Planting of safflower in western Nebraska usually is done about March 15 to April 1, probably early enough to prevent the seedling disease phase that occurred readily in the greenhouse at high temperatures.

Because such a high percentage of disease occurred in sand and nutrient solution in 9–10 days after inoculation, it seems possible that this method might be useful to differentiate between resistant and susceptible varieties; however, this method does not simulate field conditions.

SUMMARY

The causal agent of a new root rot of safflower has been determined to be *Phytophthora drechsleri*.

Soil temperatures of 25° and 30° C. were most favorable for the development of disease on safflower plants in controlled greenhouse tests; much less disease occurred at 20° and none at 17° C. These facts were closely correlated with the growth of the pathogen in culture. Total radial growth was greatest at 30°; growth at 15° C. was extremely slow.

Nearly 100 per cent of the plants inoculated at soil moisture at or above the moisture equivalent had root rot symptoms; only 57.6 per cent of those at low soil moisture had symptoms.

Radial growth of the pathogen was greatest on cornmeal agar at pH 7.0, slightly less at pH 6.0 and 8.0, considerably less at pH 5.0, and least at pH 4.0.

Plants were grown in soil and in sand and nutrient solution and inoculated. More root rot developed on sterilized substrate than on nonsterilized. Plants growing in sand supplied with nutrient solution were most succulent and most highly susceptible to root rot.

UNIVERSITY OF NEBRASKA
LINCOLN, NEBRASKA

LITERATURE CITED

1. BRAUN, ALVIN J. Phytophthora root rot of guayule. (Abstr.) Phytopath. **34**:933. 1944.
2. CLAASSEN, C. E., M. L. SCHUSTER, AND W. W. RAY. New diseases observed in Nebraska on safflower. U. S. Dept. Agr., Pl. Dis. Rptr. **33**:73-74. 1949.
3. ———, AND ALBERT HOFFMAN. Safflower production in the western part of the northern great plains. Nebr. Agr. Exp. Sta. Cir. 87 (revised). 1950.
4. ERWIN, DONALD C. Phytophthora root rot of safflower in Nebraska caused by Phytophthora drechsleri. U. S. Dept. Agr., Pl. Dis. Rptr. **34**:306. 1950.
5. GOSS, R. W. Pink rot of potatoes caused by Phytophthora erythroseptica Pethyb. Nebr. Agr. Exp. Sta. Res. Bul. 160. 1949.
6. HENDRICKSON, A. H., AND F. J. VIEHMEYER. Moisture distribution in soil in containers. Plant Physiol. **16**: 821-826. 1941.
7. PELTIER, GEORGE W., AND R. W. GOSS. Control equipment for the study of the relation of environment to disease. Nebr. Agr. Exp. Sta. Res. Bul. 28. 1924.
8. TOMPKINS, C. M., B. L. RICHARDS, C. M. TUCKER, AND M. W. GARDNER. Phytophthora rot of sugar beet. Jour. Agr. Res. [U. S.] **52**:205-216. 1936.
9. TUCKER, C. M. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp. Sta. Res. Bul. 153. 1931.

PATHOGENICITY OF PHYTOPHTHORA CINNAMOMI TO AVOCADO TREES, AND THE EFFECT OF IRRIGATION ON DISEASE DEVELOPMENT[1]

George A. Zentmyer and Sterling J. Richards

Avocado root rot, caused by the fungus *Phytophthora cinnamomi* Rands, is a serious problem in most of the avocado-producing areas of southern California. The pathogenicity of *P. cinnamomi* to avocado roots under environmental conditions favoring its development has been demonstrated (2, 4, 5). Of necessity, most of these experiments have been conducted with small seedling avocados under controlled conditions in a greenhouse or lathhouse. The availability of an experimental field planting[2] of 5-year-old budded avocado trees on the Los Angeles campus of the University of California provided an excellent opportunity to study the field development of avocado root rot. The present experiment was begun in January 1949 to find out more about the progress of the disease following infestation of field soil with *P. cinnamomi* and under different levels of irrigation, and to study the spread of the fungus from a center.

The experimental planting is on shallow soil, which presents problems similar to those in many avocado areas in southern California. The surface soil is medium-textured and permeable, but is underlaid at a depth of 10 to 24 in. by a dense clay horizon having a much lower water permeability. During seasons of high rainfall, or when excessive irrigation water is used, the shallow surface soil becomes more nearly saturated than it would if the dense clay layer were

[1] Accepted for publication August 30, 1951. Paper No. 686, University of California Citrus Experiment Station, Riverside, California.

[2] Planting developed by F. F. Halma, M. R. Huberty, A. F. Pillsbury, and E. R. Eggers, of the University of California College of Agriculture, Los Angeles, for use in studies on avocado root rot.

not present. Similar poorly drained soils are often found in commercial plantings but are not recommended for future plantings (3).

MATERIALS AND METHODS.—The absence of a natural infestation of *Phytophthora cinnamomi* in the test planting was determined by culturing root and soil samples. Root samples from each tree were cultured by the method usually employed in this laboratory for detecting the presence of *P. cinnamomi*. Pieces of small feeder roots, approximately 1 cm. long, were washed first in water and then briefly in 70 per cent alcohol, blotted on a paper towel, and planted on cornmeal agar. Ten root pieces were placed in each Petri dish of agar. *P. cinnamomi* grows out rapidly from affected roots on cornmeal agar, and its presence can be recorded 48–72 hr. after culturing. Soil samples were cultured in apples, using the method developed by Campbell (1) for isolating *P. cinnamomi* from the soil in pine stands affected with littleleaf in southeastern United States. The fungus was not found in the experimental area by either of these methods.

FIG. 1. Effect of soil infestation with *Phytophthora cinnamomi*, and of frequent irrigation, on development of avocado root rot. Trees in frequent (weekly) irrigation series: Upper, soil infested with *P. cinnamomi*, January 1949. Lower, soil noninfested. (Photographed August 1950.)

Twenty young trees, planted with 10-ft. spacing in three contoured rows averaging about 18 ft. apart on a hillside, were included in the experiment. All but three of the trees were budded to Fuerte, the principal commercial avocado variety in California; three of the trees had Ryan scions. Three different rootstocks were represented, namely, Topa-Topa, Northrup, and Waldin, the first two being Mexican types and the third a West Indian variety.

The soil around ten of the trees was infested in January 1949 by placing approximately ½ pt. of a wheat-oats culture of *Phytophthora cinnamomi* in each of four shallow holes (4–5 in. deep) 2–3 ft. from the trunk of each tree and an equal distance apart. No attempt was made to inoculate roots. A similar amount of sterile wheat-oats medium was placed around ten other trees.

In this area, as in commercial avocado-producing regions, irrigation is necessary during the dry season, which often extends from April through October. Two irrigation schedules were maintained on the plot during this period. Five trees in the infested soil and five in the noninfested soil were included in a frequent-irrigation series and watered for approximately 1 hr. once each week. The other ten trees (five in infested and five in noninfested soil) were watered on the regular biweekly irrigation schedule for the grove, which consisted of a 1-hr. irrigation every 2 weeks. A portable low sprinkler was placed under each tree, each sprinkler delivering approximately 120 gal. water/hr.

RESULTS.—The first symptoms of root rot appeared in August 1949 on one of the five trees in the group in infested soil irrigated once a week. *Phytophthora cinnamomi* was readily recovered in cultures from the dead and dying roots of this tree. By October 1949 three of the five trees in this group had definite symptoms of root rot, namely, wilting of leaves, lack of new growth, poor color of leaves, and leaf drop (Table 1); the other two trees had slight indications of the disease. None of the trees in the group in infested soil irrigated biweekly had top symptoms at that time, although many of the roots on these trees were rotted and *P. cinnamomi* was recovered from all ten of the trees in infested soil. None of the trees in noninfested soil had symptoms in October 1949, nor was the fungus recovered from the roots of these trees.

Early in 1950, approximately 1 year after the fungus had been added to the soil, two trees in the group that was irrigated biweekly had initial top symptoms

TABLE 1.—*Progress of avocado root rot following soil infestation with Phytophthora cinnamomi Rands around 5-yr.-old avocado trees in the field, under two levels of irrigation; five trees in each test*

Soil condition and frequency of irrigation	Number of trees having:						
	Top symptoms of root rot				*P. cinnamomi* isolated from roots		
	Jan. 1949	Oct. 1949	Jan. 1950	Aug. 1950	Jan. 1949	Oct. 1949	Aug. 1950
Infested [a]							
Weekly	0	3	5	5	0	5	5
Biweekly	0	0	2	5	0	5	5
Noninfested							
Weekly	0	0	0	0	0	0	2[b]
Biweekly	0	0	0	0	0	0	0

[a] Soil infested January, 1949.
[b] Fungus spread from infested area.

of root rot. By August 1950 all ten of the trees in infested soil, regardless of irrigation frequency, were visibly diseased (Fig. 1), the five of these trees in the frequent-irrigation series being much more severely affected at this time than those in the other series. The trees in the frequent-irrigation series in noninfested soil showed no harmful effects from the irrigation alone and made excellent growth. No differences were noted in responses of the different rootstocks.

Some measure of the progress of *Phytophthora cinnamomi* through the soil, under these conditions, was also obtained. In cultures made in October 1950 the fungus was isolated for the first time from trees in the row above that to which the fungus was added. The tree rows are approximately 15 ft. apart at this particular location; thus the fungus had evidently moved at least 15 ft. uphill from the time of soil infestation in January 1949 until the sampling in October 1950. This spread uphill obviously was not by zoospore dispersal; it was most likely by growth of mycelium through the moist soil and along roots, which extended at least 10 ft. from the trees by October 1950.

Soil-moisture conditions were followed in this experiment by means of tensiometers. In this and other experiments, a decrease in the rate of water use by avocado trees has been observed before typical leaf symptoms of the disease are observable. The decrease in water use is a direct reflection of a decreased ability of the trees to take up water, owing to destruction of feeder roots by *Phytophthora cinnamomi*. Where diseased trees occur in a commercial orchard and the irrigation system is designed to apply water uniformly to all the trees, the decreased rate of water use by the diseased trees results in excessively high soil-moisture conditions near these trees. This further increases the rate of damage to the diseased trees and creates conditions favorable to the spread of *P. cinnamomi* to adjacent areas.

The two years during which this experiment has been in progress have been years of subnormal rainfall for southern California; rainfall for the 1949–1950 season for the area in which the plot is located was 12.87 in., compared with a normal rainfall of 18 in.

SUMMARY

The pathogenicity of *Phytophthora cinnamomi* Rands to budded avocado trees in the field has been demonstrated, and information has been obtained on the rate of progress of avocado root rot in a field planting. The importance of irrigation practices is evident: in the infested soil the disease appeared sooner and caused more rapid damage on trees of a group given frequent (weekly) irrigations than on those of a group irrigated biweekly. No harmful effect resulted from frequent irrigation alone.

DIVISIONS OF PLANT PATHOLOGY AND OF IRRIGATION AND SOILS
UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. CAMPBELL, W. A. A method of isolating Phytophthora cinnamomi directly from soil. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 134-135. 1949.
2. CRANDALL, BOWEN S. Phytophthora cinnamomi root rot of avocados under tropical conditions. Phytopath. **38**: 123-130. 1948.
3. HUBERTY, M. R., AND A. F. PILLSBURY. Solid, liquid, gaseous phase relationships of soils on which avocado trees have declined. Proc. Amer. Soc. Hort. Sci. **42**: 39-45. 1943.
4. WAGER, VINCENT A. Phytophthora cinnamomi and wet soil in relation to the dying-back of avocado trees. Hilgardia **14**: 519-532. 1942.
5. ZENTMYER, GEORGE A., AND L. J. KLOTZ. Phytophthora cinnamomi in relation to avocado decline. Phytopath. **37**: 25. 1947.

CHESTNUT BLIGHT IN ITALY [1]

E. Baldacci and M. Orsenigo

Chestnut blight (*Endothia parasitica* (Murr.) P. J. & H. W. And.) was first noticed in Italy in 1938. Since then it has spread rapidly and infected large areas of chestnut forests. It is now present on more than 120,722 hectars (about 300,000 acres), and future losses are likely to become increasingly severe because the rate of spread, as well as the percentage of trees killed in individual stands, both appear to be increasing from year to year.

Data on the areas now known to be infected are given in table 1 and are shown graphically in figure

FIG. 1. Distribution of chestnut blight in Italy in 1950. Solid black areas are infection centers.

1. Two large centers of infection, 1) that of Piedmont-Liguria region and 2) that in the Venetian region, at Udine, were apparent by the end of 1942, and a third in the vicinity of Pistoia in Tuscany was noted in 1944. During succeeding years the infection spread rapidly in these centers and by 1948 new centers were apparent. No decrease in virulence and rapidity of spread was noted at any time, but rather a constant increase.

CULTURAL AND MORPHOLOGICAL CHARACTERS.—One or more isolations from conidia or mycelium, or both, were made from diseased trees in the Provinces of Novara, in Piedmont, and Pavia, Varese, Como, and Bergamo in Lombardy. These were cultured on po-

TABLE 1.—*Distribution of chestnut-bark disease in Italy to the end of summer, 1950*

Region and Province	Area (in hectars) of Chestnut forests[a]	Diseased forests: Verified	Diseased forests: Suspected
Piedmont			
Alessandria	14,870	14,870	----
Cuneo	91,788	----	50
Torino	26,281	----	1
Novara	25,540	----	3,000
Aosta	16,942	----	1
Vercelli	18,982	----	1
Liguria			
Genova	44,774	44,774	----
Savona	44,961	22,400	----
Lombardy			
Pavia	5,270	40	60
Como	31,317	2,741	10,000
Brescia	31,424	336	----
Varese	24,680	10,404	----
Bergamo	9,560	53	----
Venetia			
Udine	16,984	16,900	----
Treviso	3,801	----	100
Emily			
Modena	11,256	50	----
Parma	14,459	5	20
Bologna	13,245	----	5
Tuscany			
Lucca and Apuania	95,477	100	----
Pistoia	16,048	3,500	1,500
Firenze	25,274	----	1
Lazio			
Frosinone	4,986	1,300	500
Latina	1,106	400	----
Roma	12,939	----	5
Campania			
Benevento	1,455	200	----
Napoli and Caserta	13,676	150	----
Avellino	23,597	2,500	----
Salerno	21,515	----	1
Other Provinces	340,948	----	----
Totals	907,403	120,723	15,245

[a] From Fenaroli, L. : Il Castagno. Ramo Edit. degli Agric., Roma, 1945.

tato, beerwort, carrot, and Shear's white cornmeal agars in test tubes, and on Shear's white cornmeal agar in Erlenmeyer flasks and Petri dishes. Although there were some differences among the different isolates on a given medium, and within a given isolate on different media, no clear-cut, distinct cultural strains were found. In general the cultural characters agreed with those described by Shear et al.[2] for *Endothia parasitica*.

[1] Accepted for publication August 1, 1951.

[2] Shear, C. L., Neil E. Stevens, and Ruby J. Tiller, 1951. Endothia parasitica and related species. U. S. Dept. Agr. Bul. 380. 1917.

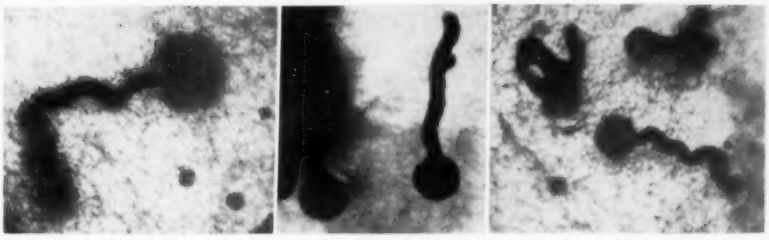

FIG. 2. Perithecia of *Endothia parasitica* (Murr.) P. J. & H. W. And. in culture. At left, a perithecium with two necks.

Pycnidiospores on chestnut bark measured 2.30-6.90 (average 3.36) μ long and 0.69-2.30 (average 1.57) μ wide; pycnidiospores in culture had the same size but averaged 3.77 μ long and 1.60 μ wide. Ascospores on chestnut bark measured 0.64-12.1 (average 9.0) μ long and 2.70-5.40 (average 4.15) μ wide. These measurements agree closely with the averages reported by Shear et al. for *Endothia parasitica*. This supports the conclusion, from cultural studies, that the strains of the fungus found in Italy are the same as those found in America and Asia.

A culture isolated from diseased trees in Pavia, Lombardy, sent to Dr. G. F. Gravatt, Division of Forest Pathology, U. S. Department of Agriculture, Beltsville, Maryland, was considered by him to be identical with American strains. A specimen of diseased trees was also sent, on which the following comments were received by us from Dr. Gravatt: "The specimen of *Endothia parasitica* collected in Italy, at Ponte Nizza, Lombardy, has been cultured by John Flannagan of this Division. His cultures appear to be typical for the characteristics of *Endothia parasitica*, based on a study of cultures made from material from Asia and [the United States]. The pycnidial growth and spores appear to be typical. No ascospores have been produced."

PERITHECIA IN CULTURE.—Perithecia developed in pure culture isolated from Albavilla, Como (Lombardy), and grown on test-tube slants of beerwort agar kept 7–10 months at room temperature (approx. 18–24°C.) and protected from light. These perithecia were black, membranaceous, typically globose, 150–230 (average 180) μ wide, with a neck 45–90 μ wide and 380–730 (average 590) μ long; the long necks were cylindrical, and straight, curved, or undulated. Some perithecia were irregular in shape and had two or three necks. The perithecia were sunken into the medium, but the necks protruded. No ascospores were found in these perithecia. This is the first report of perithecia produced in culture by *E. parasitica* (Fig. 2).

SUMMARY

The chestnut bark disease (chestnut blight) was first observed in Italy in 1937, and in the intervening years it has spread rapidly and far. At the end of summer, 1950, the area of known diseased chestnut forests amounted to 120,722.95 hectars (about 300,000 acres) with some additional thousand of hectars possibly being infected. The disease now involves approximately 20 per cent of the chestnut forests of those Provinces where it now is present, or about 15 per cent of the entire chestnut area in Italy. In those areas in which the disease has been present for some time, the mortality is high, and in the newer areas it is increasing.

Detailed study of cultural characters of numerous isolates from different regions, and of shape and size of conidia and ascospores, prove rather conclusively that the strains of the fungus present in Italy are essentially the same as those found in America and Asia.

Perithecia formed by *E. parasitica* in pure culture are reported for the first time.

INSTITUTE OF PLANT PATHOLOGY, UNIVERSITY OF MILAN, MILAN, ITALY

THE EVOLUTION OF SUSCEPTIBILITY TO TOBACCO MOSAIC IN NICOTIANA AND THE ORIGIN OF THE TOBACCO MOSAIC VIRUS [1]

W. D. Valleau

The numerous varieties of *Nicotiana tabacum* are susceptible to the tobacco mosaic virus (TMV), with the exception of Ambalema and related varieties from Colombia and the synthetic varieties developed by the addition of the necrotic spotting *N* factor from *Nicotiana glutinosa*. Many species of *Nicotiana*, as *N. glutinosa*, *N. rustica*, *N. gossei*, *N. repanda*, and *N. suaveolens*, carry the dominant *N* factor, which makes them practically immune to TMV. Other species, as *N. sylvestris* and *N. tomentosa*, react by mottling as do the cultivated tobaccos.

It seems probable that all of the species of *Nicotiana*, and perhaps some other members of the Solanaceae, originated from one ancestor in the distant past. It is therefore of interest to speculate on the genetic make-up of this progenitor so far as its reaction to TMV is concerned. While we know nothing about the plant from direct observation, enough is known about the modern species of *Nicotiana* so that more than a guess can be made with respect to the genetic make-up of the progenitor. It is the purpose of this paper to show that the present species of *Nicotiana* with their peculiar reactions to TMV could and probably did evolve in the absence of TMV. This necessarily means that TMV entered the picture in relatively recent times, and a suggestion is made as to its probable origin.

Genetics of resistance and susceptibility.—The modern species of *Nicotiana*, with respect to their reactions to TMV, carry certain genes by which they may be characterized. These are *N*, *N'*, *n'*, *A*, and *a*. *N* causes necrotic spotting with all strains of TMV. *N'* causes a necrotic reaction to the English aucuba mosaic virus, certain American strains of TMV, and the plantago virus, but no necrosis with most strains of TMV, and so plants with *N'* react by mottling. *N'* is found in *N. sylvestris* and perhaps in other susceptible species but is also found in many varieties of tobacco. The *n'* gene fails to cause necrosis with any strain of TMV or the plantago virus but causes mottling with all strains. It is found in many varieties of *N. tabacum*. It is probable but not positively proved that *N*, *N'*, and *n'* are alleles. The *A* gene, found in all varieties of *N. tabacum* except Ambalema and in many wild species, is the gene responsible for susceptibility or mottling of *Nicotiana* when inoculated with TMV. An allele of *A* is the recessive *a* found in the variety Ambalema, presumably in *N. tomentosiformis* which has the same type of resistance to TMV as Ambalema, and perhaps in other species. This gene, in a homozygous condition, results in what appears to be true resistance to TMV.

Progenitor of Nicotiana.—With this background of facts and the knowledge that mutations are almost always in the direction of dominant to recessive, rarely if ever in the reverse, we may approach the genetic make-up of the progenitor of *Nicotiana* with respect to its reaction to TMV. The dominant *N* and *A* factors are both present in *N. glutinosa* and probably in many other wild species of *Nicotiana*. Unless we assume that in each of these species mutations have taken place that resulted in these dominant genes, we must assume that they were derived from some progenitor. We may then logically assume that the parent species was a plant practically immune to TMV because of the *N* factor, but potentially highly susceptible to TMV because of the *A* factor. Following this line of reasoning it is probable that the same ancient plant may have been the progenitor of other genera of the Solanaceae, as mosaic susceptibility is common to many genera, and an *N* factor is found in potatoes, in *Datura*, and in other related genera. If this view is correct, the tobacco mosaic virus could not have existed as a cause of disease in the early form of *Nicotiana*. It must have had some other host.

Origin of susceptibility.—If the progenitor of *Nicotiana* was practically immune to TMV, how then have the susceptible species developed and why are nearly all of the cultivated varieties of tobacco highly susceptible to the virus? This can probably best be answered by looking into the genetic make-up of two wild species that have been studied sufficiently so that we know something of their genetic make-up with respect to their reactions to TMV. The first of these is *N. glutinosa*. It is well known that it carries the *N* factor for practical immunity to TMV, and that this factor when superimposed on the *AAAA* factors for susceptibility to tobacco mosaic results in practically immune varieties of *N. tabacum*. McKinney and Clayton (4) have shown that inoculated plants of *N. glutinosa* develop typical mottling if grown at about 97°F., while Valleau (5) demonstrated that susceptibility in *N. glutinosa* is due to an *A* factor distinct from *N*. When this *A* factor is transferred to *N. tabacum*, susceptibility is introduced into an otherwise resistant variety that originally carried the Ambalema resistance factors. Therefore, *N. glutinosa*, in common with the postulated progenitor of *Nicotiana*, carries factors for resistance and susceptibility to TMV.

The other species that should be examined is *N. sylvestris*, a wild species highly susceptible to mosaic. It and another susceptible species, *N. tomentosa*, prob-

[1] Accepted for publication August 4, 1951.
The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

ably entered into the make-up of the amphidiploid *N. tabacum*. The genetic make-up of *N. sylvestris*, with respect to reaction to TMV, is nearly identical with that of *N. glutinosa* except that the *N* factor in *N. glutinosa* is represented by the *N'* factor in *N. sylvestris*. When *N. sylvestris* is inoculated with an ordinary strain of TMV, mottling develops; but when it is inoculated with the English aucuba mosaic virus (3) or several other American strains of TMV (6) or with the plantago virus, necrotic spots develop as in *N. glutinosa*. Many varieties of *N. tabacum* carry the *N'* factor. Other varieties of *N. tabacum*, when inoculated with the viruses that cause necrotic spotting in *N. sylvestris* or in *N'N'* varieties of tobacco, respond with mottling. In these varieties either *N'* has been lost completely or it has mutated further to the recessive *n'* gene. Thus there appears to be a series of alleles *N*, *N'*, and *n'*. Mosaic-susceptible species could then have originated from mosaic-resistant species by mutation without TMV playing any part in the process. As presumably all of the cultivated varieties of *N. tabacum*, with the possible exception of Ambalema, have been derived from an amphidiploid of two susceptible species, there is no chance that any of them could be resistant to TMV except possibly by mutation of the *A* gene to *a*, if this is the way *a* originated, but then *a* probably would have been lost because of the three other dominant *A* factors.

Geographic distribution of resistance factor.—If the species of *Nicotiana* have evolved, so far as their reaction to mosaic is concerned, in the way indicated, then we would expect to find, in widely distributed wild species of *Nicotiana*, species that are practically immune, others that are made up of immune, mixed, and susceptible families, and others that are susceptible, on each continent where *Nicotiana* is native. In the area in western South America, considered to be the center of development of the genus, it would be expected that even though a mutation from *N* to *N'* or *n'* took place in a species it might be difficult for the mutant to survive, because of cross pollination with plants homozygous for the dominant *N* factor. However, if heterozygous seeds were carried into an isolated area, by birds or other means, a heterozygous population would be expected to develop, and in the process of spread to other isolated areas in the vicinity homozygous susceptible populations could be isolated. Species with resistant, mixed, and susceptible populations are illustrated by *N. alata* and *N. undulata*. In Texas, *N. repanda*, an *N* species, is widely distributed. In other parts of western North America several mosaic-susceptible species are found.

In Australia there are several species that differ from the American species in having quite different chromosome numbers, suggesting isolation from the American species for a very long time. At least four of the Australian species, according to unpublished results of Diachun and Valleau, carry the *N* factor. These are *N. gosseii*, *N. suaveolens*, *N. maritima*, and *N. velutina*. It is probable that these species also are represented by both resistant and susceptible families, as Holmes (2) reports that the Australian species he tested, including these four, respond by mottling. Thus there are both resistant and susceptible species of *Nicotiana* on every continent where *Nicotiana* is native.

Origin of tobacco mosaic virus.—If the evolution of *Nicotiana* species has taken place without aid of TMV, when did this virus enter the picture? There is not enough historical material on tobacco mosaic to indicate when it was first seen as a disease in tobacco. It is interesting, however, that a disease as conspicuous and as destructive as tobacco mosaic was not mentioned in American literature for nearly 400 years, in spite of the fact that tobacco was raised by well educated, keen observers and was for many years virtually legal tender.

It is probable that a mosaic disease of tobacco first occurred when tobacco was grown by man in the vicinity of *Plantago* sp. infected with the plantago virus or in the vicinity of some other as yet undiscovered virus in some other genus. On several occasions we have observed tobacco plants from a plant bed surrounded by *Plantago*, affected by a virus that caused systemic necrosis in *N'N'* varieties but when transferred to *n'n'* varieties caused mottling typical of a distorting strain of TMV. The virus was determined to be the plantago virus (7).

Since the plantago virus frequently causes a mosaic disease of tobacco (7), and since the virus withstands drying and so could maintain itself in tobacco from year to year, and since plant viruses are known to mutate at a relatively rapid rate, the obvious conclusion is that the plantago virus must have become established in cultivated tobacco and so is the most probable progenitor of the numerous strains of TMV.

Plantago is an almost universal genus but it is not known whether the virus is coextensive with it. Therefore it cannot be determined at present upon which continent the disease of tobacco originated or whether it has had a multiple origin.

Discussion.—The question as to how resistance or susceptibility to a virus disease of plants has originated and the part which the virus played in the evolution of a genus is of some scientific interest. In the present paper an attempt has been made to show that everything that has taken place in the genus *Nicotiana*, with respect to practical immunity, resistance, and susceptibility, could and probably did take place in the entire absence of the tobacco mosaic virus. The only part for which the virus was really necessary was in the study of the reaction of the various species and varieties to infection. Unless it is assumed that mutations resulting in dominant factors are common in *Nicotiana*, it must be admitted that the two dominant genes—*N* for necrotic spotting and *A* for mosaic susceptibility—were both present in the progenitor of the numerous *Nicotiana* species. Then by mutation of the *N* factor, plants have resulted in which necrosis does not occur and we have the origin of susceptible species, all without the intervention of TMV.

In contrast with this process, Holmes (2) has postulated that in some way TMV played a part in the development of resistant species, and that in the center of the greatest number of resistant species the original home of the virus could probably be found. This hypothesis is based on several assumptions: 1) that there is some relationship, from an evolutionary standpoint, between the virus and the plant for which it was named; 2) that TMV is to be found in certain of the wild species of *Nicotiana* in the dry mountain areas of western South America; 3) that there is some mechanism by which this virus is readily carried long distances so that it may play its part in the process of evolution (1)[2]; 4) that the virus is capable of eliminating highly susceptible species, although those moderately tolerant will survive; 5) that in some way the presence of the virus influences the development of resistance in the genus, although the mechanism is not made clear; and 6) that originally there must have been susceptible species, in which the virus developed, and resistant species of the hypersensitive type which remained after the susceptible species had been eliminated by the virus.

In the evolutionary process described in the present paper these assumptions are not necessary in order to have a clear picture of the process by which susceptible species and varieties have developed from practically immune species. The only assumptions are that two mutations of the *N* gene have occurred, and possibly one mutation of the *A* gene. If these mutations are admitted as possibilities, there is a satisfactory explanation for the reactions to TMV of the known species and varieties of *N. tabacum*.

If *N. tabacum* as a cultivated species did not exist, it is probable that the tobacco mosaic virus would not now be known to science in spite of the more than fifty other species of *Nicotiana* in existence.

When a susceptible cultivated species is grown in the vicinity of a virus to which it has never before been exposed, and a vector is present, the virus may immediately be transferred to the cultivated plants, and a disease result. If the plant happens to carry a factor for necrotic spotting and no disease results, the plant is immune; but the virus has played no part, so far as evolution is concerned, with respect to either susceptibility or resistance.

It is not inconceivable, then, that tobacco made its first contact with TMV when it was first cultivated by man in the vicinity of plantains infected with the plantago virus. If the variety happened to be *n'n'*, a mottling disease fairly typical of TMV would have resulted. As the plantago virus withstands drying, it could have been carried over to the next season's crop and a disease of tobacco would have been well on its way toward becoming established. Infection from dry tobacco has undoubtedly aided in the isolation and perpetuation of strains of TMV, because when the hands become contaminated with virus from dry tobacco there is always the chance that a mutant strain will be transmitted in a pure form to a tobacco seedling; whereas in transmission from living plants the original strain predominates. Once the plantago virus has become established in cultivated tobacco, mutations over many years could readily account for the numerous strains now known, and for the differences now recognized between some of the strains of TMV and the plantago virus.

Considering the wide host ranges of plant viruses, it is questionable whether any significance, from an evolutionary standpoint, should be attached to a virus-host relationship, particularly when the host is a cultivated plant that does not exist as a wild species.

[2] In reading Goodspeed's Plant Hunters in the Andes, one is impressed by the amount of hunting and travel that was necessary in order to find specimens of *Nicotiana*. Sometimes only one or two plants of a species could be found in several days' search. In other limited areas there were sometimes many specimens of a species but usually separated widely from other species. It may be significant that Goodspeed does not mention having found mosaic in any of the wild susceptible species observed.

CONCLUSIONS

The reactions of modern species of *Nicotiana* to the tobacco mosaic virus can all have originated by mutations of the dominant *N* and *A* genes, present in a practically immune *NNAA* progenitor of the present species of *Nicotiana*, without the intervention of the mosaic virus. It is probable that TMV first made its appearance as a disease of tobacco when tobacco was grown in close proximity to the plantago virus. Gradual mutation of the virus in tobacco could account for the differences between TMV and the plantago virus and for the numerous strains of TMV now recognized.

KENTUCKY AGRICULTURAL EXPERIMENT STATION
LEXINGTON, KENTUCKY

LITERATURE CITED

1. GOODSPEED, T. HARPER. Plant hunters in the Andes. 429 pp. Farrar and Rinehart. 1941.
2. HOLMES, FRANCIS O. Indications of a New-World origin of tobacco-mosaic virus. Phytopath. **41:** 341-349. 1951.
3. KUNKEL, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. **24:** 437-466. 1934.
4. MCKINNEY, H. H., AND E. E. CLAYTON. Genotype and temperature in relation to symptoms caused in Nicotiana by the mosaic virus. Jour. Hered. **36:** 323-331. 1945.
5. VALLEAU, W. D. The genetics of mosaic resistance in Nicotiana glutinosa. Jour. Agr. Res. [U.S.] **78:** 77-79. 1949.
6. ———. Localization and resistance to tobacco mosaic, in Nicotiana. Ky. Agr. Exp. Sta. Bul. **360:** 202-230. 1935.
7. ———, AND E. M. JOHNSON. An outbreak of Plantago virus in burley tobacco. Phytopath. **33:** 210-219. 1943.

RESISTANCE TO DOWNY MILDEW IN LIMA BEAN, AND ITS INHERITANCE[1]

H. Rex Thomas, Hans Jorgensen, and R. E. Wester

Downy mildew, caused by *Phytophthora phaseoli* Thaxt., has occurred sporadically on lima beans in the Atlantic Coast States since it was first described by Thaxter in Connecticut in 1889.[2] Although experiments have shown that the disease can be reduced by proper applications of fungicides, no general control program has been followed by growers, chiefly because of the sporadic occurrence of the disease and the low margin of profit from the crop.

Because the disease has been serious every year since 1945 (except 1947) in large canning and freezing areas in New Jersey, Delaware, and Eastern Shore, Maryland, a collection of foreign and domestic lima beans was tested to find resistance to downy mildew.

Herein are reported the occurrence of resistance in lima beans and its inheritance as indicated by the behavior of the F_1, F_2, F_3 backcross and outcross populations to inoculation with the downy mildew fungus.

MATERIALS AND METHODS.—The fungus used for the inoculations was isolated from an infected pod of Fordhook lima bean collected at Bridgeton, New Jersey, September 1947. In the greenhouse young seedlings with primary leaves just unfolding were sprayed with a water suspension of spores obtained from infected plants. The plants were kept in a moist chamber 4–6 days after inoculation, until the fungus sporulated. In the field the fungus was established by spraying susceptible plants scattered throughout the plot with spores from lima-bean agar cultures. The plants were kept moist by frequent overhead irrigations.

The screening of the various lima-bean collections for their reaction to downy mildew and all of the inoculations in the inheritance studies were made in the greenhouse at Beltsville, Maryland, on young seedlings. The resistant lines selected as parental material were further tested in the field at Beltsville, Maryland; Long Island, New York; and Newark, Delaware, to determine whether greenhouse tests were adequate to evaluate field resistance as well as to observe the resistance of the pods to downy mildew infection.

In the greenhouse plants were grown in flats or 4-in. pots, except for certain F_2 families saved for F_3 seed and planted in 10-in. pots. Only a limited number of F_2 plants were grown to maturity in the greenhouse for seed production.

SOURCE AND DEGREE OF RESISTANCE.—Among 113 foreign and domestic lima-bean collections, four lines possessed resistance in a high degree: 1) 4913[3], or P.E.I.[4] 164155 from India; 2) 4981, or P.E.I. 163580 from Guatemala; 3) 8747, a selection from southeastern United States; and 4) 4931, line L-37 from R. W. Allard of the University of California. A fifth line, 49003, a wild lima type from Mexico previously reported highly resistant, is now considered to be less resistant than the others.[5]

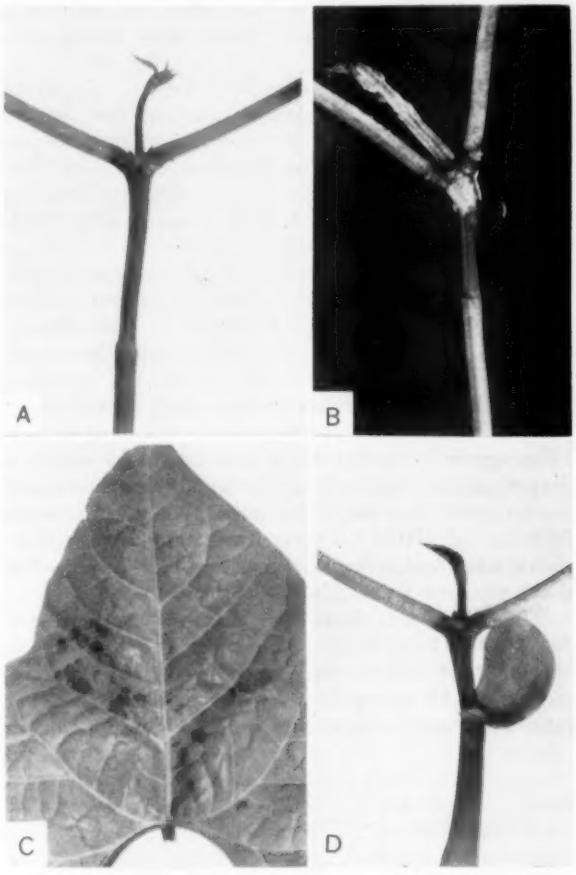

FIG. 1. Reaction of susceptible and resistant lima beans to downy mildew inoculation. A. Healthy noninoculated Triumph. B. Abundant sporulation on susceptible Triumph. C. Necrotic lesions on leaf of resistant parent 4931. D. Necrotic streaking on stem of resistant parent 4931.

In the field plots at Beltsville, Maryland, no infection has been observed on the pods or leaves of the resistant plants. In the cooperative tests with H. S. Cunningham at Riverhead, Long Island, New York, and R. S. Cox and J. W. Heuberger at Newark, Delaware, in 1950, a few infected leaves with a little sporulation were found on a few of the resistant lines.

Necrotic streaking of the stem and frequent killing of the growing point occurred on young resistant seed-

[1] Accepted for publication August 27, 1951.

[2] Thaxter, R. A new American *Phytophthora*. Bot. Gaz. 14: 273-274. 1889.

[3] Local accession number.

[4] Refers to accession number of Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[5] Thomas, H. Rex, Hans Jorgensen, H. H. Fisher, and R. E. Wester. Resistance to downy mildew in lima beans. (Abstr.) Phytopath. 41: 566-567. 1951.

lings inoculated in the greenhouse under conditions very favorable for disease development (Fig. 1, D). Less frequently, necrotic spots 3–4 mm. in diameter developed on the inoculated primary leaves of resistant plants (Fig. 1, C). A limited amount of sporulation occurred on this necrotic tissue.

Under moist greenhouse conditions the fungus sporulated abundantly on the susceptible plants, usually 4–6 days after inoculation (Fig. 1, B). The plants rapidly collapsed and usually were dead 2–3 days later. On older plants in the field, most sporulation occurred on the pods. Young foliage was killed but very little sporulation occurred; older leaves were usually not infected.

Reaction of selfed parents.—Ten to 15 plants of each of the selfed parents used in the various crosses were inoculated with the downy mildew fungus. The varieties Concentrated Fordhook, Fordhook 242, Thorogreen, Early Thorogreen, and breeding line No. 8112 were susceptible. The other parents, 8747, 4913, 4981, and 4931, were resistant.

Reaction of F_1.—Forty-two F_1 plants from the following crosses were inoculated with the downy mildew fungus: Fordhook × 4913, Fordhook × 4981, Thorogreen × 4981, Thorogreen × 4913, and Thorogreen × 8747. All were resistant.

Reaction of backcross and outcross populations.—When 83 plants from the backcross Thorogreen × (Thorogreen × 4913) were inoculated, 44 were resistant and 39 susceptible. Assuming that resistance is controlled by a single dominant gene, the deviation from the calculated 1:1 ratio of resistant to susceptible plants was nonsignificant with a χ^2 of 0.301, well below the 5 per cent point, 3.841.

When 27 plants from the outcrosses of Fordhook 242 × (8112 × 4913) and Concentrated Fordhook × (8112 × 4913) were inoculated, 16 were resistant and 11 susceptible. This deviation from a 1:1 ratio was nonsignificant with a χ^2 of 0.926.

Table 1.—*Reactions of F_2 progenies when inoculated with Phytophthora phaseoli*

Parentage of cross	Number of plants inoculated	Number of plants		χ^2 for 3:1 ratio[a]
		Resistant	Susceptible	
Thorogreen × 4931	98	75	23	0.122
Thorogreen × 4913	93	69	24	0.032
Thorogreen × 4981	130	104	26	1.733
Thorogreen × 8747	122	89	33	0.275
Total	443	337	106	0.272

[a] 5 per cent point, 3.841.

Reaction of F_2.—The inoculated F_2 plants from each of the four crosses (Table 1) segregated into a ratio of 3 resistant to 1 susceptible plant, indicating a single-factor difference. The χ^2 values showed that the observed data fit the calculated rather closely for the four crosses. Of a total of 443 plants (for all four crosses), 337 were resistant and 106 susceptible. The χ^2 value of 0.272 is well below the 5 per cent point, 3.841.

Reaction of F_3.—Of 84 F_3 families derived from resistant F_2 plants, 35 were homozygous resistant and 49 were heterozygous when inoculated with the downy mildew fungus (Table 2). The deviation from the calculated 1:2 ratio of homozygous to heterozygous resistant families was nonsignificant with a χ^2 of 2.4. In the 49 heterozygous families the segregation of total resistant to total susceptible plants showed a poor fit to a 3:1 ratio. Accumulated χ^2 for the 49 families, however, showed a nonsignificant departure from a 3:1 ratio. The progenies from five F_2 selfed families of the susceptible plants were homozygous for susceptibility.

Table 2.—*Reaction of F_3 families descended from previously tested F_2 plants, when inoculated with Phytophthora phaseoli*

Classification in F_3 generation	Number of families tested[a]	Number of plants tested	Number of plants	
			Resistant	Susceptible
Homozygous resistant	35	1065	1060	5
Heterozygous resistant				
Observed	49	1540	1209	331[b]
Calculated 3:1 ratio			1155	385
Homozygous susceptible	5	131	0	131

[a] χ^2 for 1:2 distribution of families was 2.400; 5 per cent point = 3.841.

[b] χ^2 for total populations was 10.098; 5 per cent point = 3.841. Accumulated χ^2 was 47.312; 5 per cent point = 66.102 for 49 degrees of freedom.

Discussion.—These results demonstrate that in the lima-bean hybrids investigated the inheritance of resistance to downy mildew infection is controlled by a single dominant genetic-factor difference. The deficiency of susceptible plants in the 49 F_3 heterozygous families probably was due to failure of the inoculation to eliminate all susceptible plants. The five susceptible plants recorded in the homozygous resistant F_3 families may have resulted from seed mixture or from misclassification of plants with slight sporulation in the susceptible instead of the resistant class.

The nature of the resistance within the resistant lines has not been studied. The development of some necrosis on the susceptible lines accompanied by abundant sporulation, and considerable necrosis and little or no sporulation on the resistant lines, suggests a parallel with the type of resistance observed in certain potato varieties resistant to the late blight fungus, *Phytophthora infestans* (Mont.) DBy.[6] If this comparison is correct, strains of the downy mildew organism which are more pathogenic on the present resistant lines of lima bean might be found, as has occurred in the potato late blight fungus.

[6] Müller, K. O., and Lothar Behr. Mechanism of *Phytophthora*-resistance of potatoes. Nature 163: 498-499. 1949.

SUMMARY

Resistance to downy mildew of lima beans was found in four lima-bean collections. In the field, very little sporulation of the fungus has been observed on the resistant lines. In the greenhouse young plants of resistant lines upon inoculation may develop necrosis, accompanied sometimes by limited sporulation.

The response to inoculation with the downy mildew fungus of the F_1, F_2, F_3, and F_1 plants backcrossed and outcrossed to susceptible commercial parents showed that resistance is controlled by a single dominant gene.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

MECHANISM OF INHIBITION OF PLANT VIRUS INFECTION BY FUNGAL GROWTH PRODUCTS[1]

B. M. Gupta and W. C. Price

The mechanism of inhibition of plant virus infection by materials such as normal serum (8, 26), trypsin (23, 27), ribonuclease (19, 24), sap of pokeweed (10) and of spinach (13, 21), fungal growth products (11, 16, 17), milk (8, 11, 17), and insect juices (3, 15) is still a subject of controversy. On the one hand it is held that these materials enter into a reversible combination with virus and thus exert their inhibitory action by affecting the virus itself. On the other hand it is held that the inhibitors have an effect on cells of the host plant, rendering them less susceptible to infection, and thus have no direct effect on the virus. Because the inhibitory substance from each of these sources acts immediately on mixing with virus and because virus activity can subsequently be regained by dilution, treatment with enzymes, high-speed centrifugation, or ultrafiltration, it seems likely that the mechanism of inhibition for all these substances is similar, if not identical.

In a recent paper (14) the writers showed that 41 of 49 species of fungi produced materials capable of inhibiting infection with the virus of tobacco mosaic, tobacco necrosis, or Southern bean mosaic and that 10 did so to the extent of 85 per cent or more. Experiments have now been carried out with some of the latter species, particularly with *Trichothecium roseum* Link, to determine whether the inhibition results from an effect on the host or on the virus. The evidence obtained strongly supports the hypothesis that it is due to action on the host.

REVIEW OF THE LITERATURE.—Lojkin and Vinson (23) thought that the inhibitory effect of trypsin on tobacco mosaic virus infection was due to its hydrolytic activity. However, Caldwell (6) found that a noninfectious mixture of trypsin and aucuba mosaic virus could be rendered infectious by heating to 70° C. to destroy the trypsin. Moreover, Stanley (27) showed that the inhibitory effect of trypsin occurred at pH values at which trypsin is not proteolytically active and that virus infectivity could be regained by digesting the trypsin with pepsin. He also found that the inhibitory effect of trypsin on tobacco mosaic virus infection was greater when *Phaseolus vulgaris* L. was used as a test plant than when *Nicotiana glutinosa* L. was so used. A similar result was later obtained by Loring (24) in studies on the inhibitory effect of ribonuclease. The results led Stanley to conclude that trypsin has no direct action on the virus but produces its effect by altering susceptibility of the host. On the contrary, work with potato virus X led Bawden and Pirie (2) to suggest that loss of infectivity might result from formation of a loose, easily broken virus-trypsin complex.

Chester (8) states that normal serum and nonspecific immune sera caused an approximately constant percentage reduction in infectivity at all dilutions of virus, whereas specific antiserum caused a higher percentage loss in infectivity at high dilutions of virus than at low dilutions. Furthermore, the neutralizing effect of specific immune serum increased with length of contact with virus, whereas the full effect of normal serum and of nonspecific immune serum was obtained at once. Chester concluded that normal sera and nonspecific immune sera reduced infectivity by rendering the host less susceptible.

Hamilton (15) observed that addition of juice from crushed insects to Hyoscyamus III virus rendered the mixture noninfectious and suggested that the juice had a deleterious effect on the virus. Black (3), on the other hand, obtained evidence that insect juices alter susceptibility of the host plant. He found that extracts of clover leafhoppers inhibited infection with potato yellow dwarf, tobacco mosaic, potato X, turnip mosaic, tobacco necrosis, and tobacco ringspot viruses and that juice of various insects, including aphids, leafhoppers, and mosquitoes, inhibited tobacco mosaic virus infection. The inhibitor, not readily dialyzable, destroyed in both acid and alkaline solutions and by heating to 70° C. or higher, and stable at room temperature over a 24-hr. period, caused visible burning of leaves to which it was applied in concentrated solutions. The virus could be separated from it by ultracentrifugation or by ultrafiltration.

[1] Accepted for publication August 30, 1951.

Based on a thesis submitted by the senior author in partial fulfillment of the requirements for the Doctor of Philosophy degree.

This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

Contribution from the Plant Virus Laboratory of the University of Pittsburgh.

Johnson (17) found that milk, blood serum, citrus fruit extracts, and growth products of *Aerobacter aerogenes* and *Aspergillus niger* are capable of inhibiting infection with tobacco mosaic virus. He found no evidence that any of these materials, or trypsin, or other tested materials, modify susceptibility of the host plant and suggested that inhibition was due to a loose, easily broken, molecular union between virus and inhibitor. This conclusion was supported by Fulton (11), who studied the effect of trypsin, milk, bovine serum, and other materials on potato ringspot, cucumber mosaic, tobacco ringspot, and bean mosaic viruses.

Takahashi (28, 29) describes the isolation of a material, believed to be a polysaccharide, from yeast which was capable of inhibiting tobacco mosaic virus infection. He believed that inhibition was brought about by a reaction between the inhibitory material and a group on the virus particle necessary for infectivity.

Kuntz and Walker (21) found that a component present in an extract of spinach leaves was capable of inhibiting infection of cabbage mosaic virus on *Nicotiana glutinosa* but not of tobacco mosaic virus on the same host. They concluded that the effect of the inhibitor was on the virus.

It was shown by Kleczkowski (18) that ability to combine with and precipitate tobacco mosaic virus was not correlated with ability to inhibit infection. Presumably, combination with virus is not important in preventing infection unless the combination occurs with a specific group on the virus particle responsible for activity.

MATERIALS AND METHODS.—*Preparation of fungus extracts.*—Extracts used in studies to be reported were obtained from fungus cultures grown on a medium prepared in the following manner. Cornmeal, 40 gm. in 500 ml. of water, was boiled for 30 min. and then filtered through a double thickness of cheesecloth. The filtrate was added to 20 gm. of dextrose and 4 gm. of bacto-beef (Difco) and the volume brought up to 1 liter with water. About 200 ml. of medium were transferred to each of a number of penicillin flasks and sterilized at 15 lb. pressure for 20 min.

Fungi from standard agar slants were transferred to the flasks and grown for about 3 weeks at a temperature between 22° and 28° C. The contents of the flask were well shaken and ground in a mortar, and the fluid was pressed out through two layers of cheesecloth. The extract was centrifuged at 3000 r.p.m. for 15 min. or, in other cases, passed through a Seitz filter. The supernatant or filtrate was used as a test solution.

Viruses.—Tobacco mosaic (TM) and tobacco necrosis (TN) viruses were maintained by frequent transfers to plants of Turkish-type tobacco (*Nicotiana tabacum* L.) grown in a greenhouse. Southern bean mosaic (SBM) virus was maintained in the Bountiful variety of garden bean (*Phaseolus vulgaris* L.)

Local-lesion hosts.—Plants of the Kentucky Wonder variety of garden bean, a local-lesion host, were used in testing activity of SBM virus. Similarly, plants of the Golden Cluster variety of garden bean, of *Nicotiana glutinosa* L., or of Holmes' necrotic-type tobacco (a derivative of *N. tabacum* × *N. digluta* Claussen & Goodspeed), were used to test infectivity of TM and TN virus samples. Plants were grown in 4-in. pots, in a greenhouse held at 70° F. or above, to a stage suitable for inoculation.

Method of testing inhibitory activity.—Inhibitory activity of a fungal extract was tested by mixing it with virus in a test tube or by application to test plant leaves, followed by inoculation. Inhibitory activity was judged by counts of the local lesions produced in comparison with those produced by an appropriate control. Both the control solution and that containing the inhibitor were buffered by addition of sufficient 1.0 M phosphate buffer to bring the molality to 0.1 at pH 7. Activity of the inhibitor, expressed as percentage reduction in lesion counts, was calculated by dividing the number of lesions produced by the test solution by the number produced by the control, multiplying the quotient by 100, and subtracting the product from 100.

EFFECT OF CONCENTRATION CHANGES ON INFECTIVITY.—There are two theories to account for the sigmoidal shape of virus infectivity-dilution curves. One of these states that infection can be induced by a single virus particle and that the probability of obtaining infection is thus the probability of finding one or more virus particles in the small volume of inoculum that comes into contact with a susceptible region of the host (22, 30). This theory is described by the equation:

$$Y = N(1 - e^{-vnx}) \qquad (1),$$

where Y is the expected number of infections, N is the number of susceptible regions, x is the dilution factor, v is an elemental volume, and n is the number of virus particles in unit volume of the undiluted inoculum.

The second theory states that susceptible regions of the host vary in susceptibility in such a way that the logarithm of the minimum virus concentration necessary to cause infection is normally distributed (5, 20). This theory can be described by the equation:

$$Y = \frac{N}{\sigma\sqrt{2\pi}} \int_{-\infty}^{t} \exp\left[-\tfrac{1}{2}\left(\frac{t-\xi}{\sigma}\right)^2\right] dt \qquad (2),$$

where Y is the expected number of infections, N is the mean number of susceptible regions, $t = \log_{10} x$ where x is the actual virus concentration, $\xi = \log_{10} x^0$ where x^0 is the virus concentration that causes 50 per cent of the susceptible regions to become infected, and σ is the standard deviation.

If an inhibitor affects susceptibility of the host, then N in both equations 1 and 2 would be expected to vary with the concentration of the inhibitor. If, on the other hand, the inhibitory substance affects the virus, then the value of n in equation 1 and the value of t in equation 2 would vary with the concentration of inhibitor. The mathematical approach to solution of the present problem was to see whether the experimental data could best be interpreted on the assumption that the number of susceptible regions (N) is

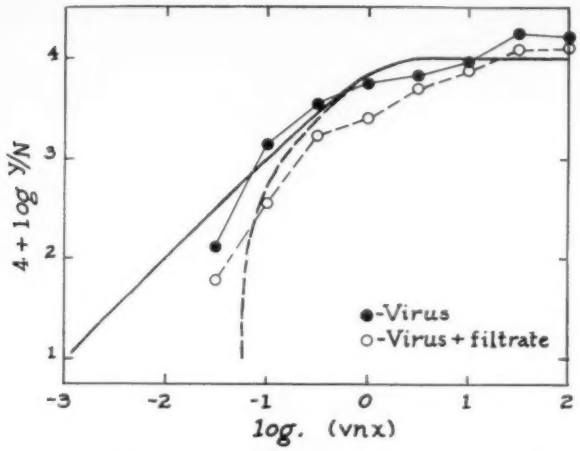

Fig. 1. Infectivity-dilution curves for SBM virus on Kentucky Wonder bean leaves with and without a constant amount of inhibitor from *Trichothecium roseum*. The heavy solid line represents the theoretical curve expected for virus alone, the light solid line the experimental one. The heavy broken line represents the theoretical curve expected if a constant amount of inhibitor inactivates a constant amount of virus. The light broken line is the curve obtained with a mixture of virus and inhibitor.

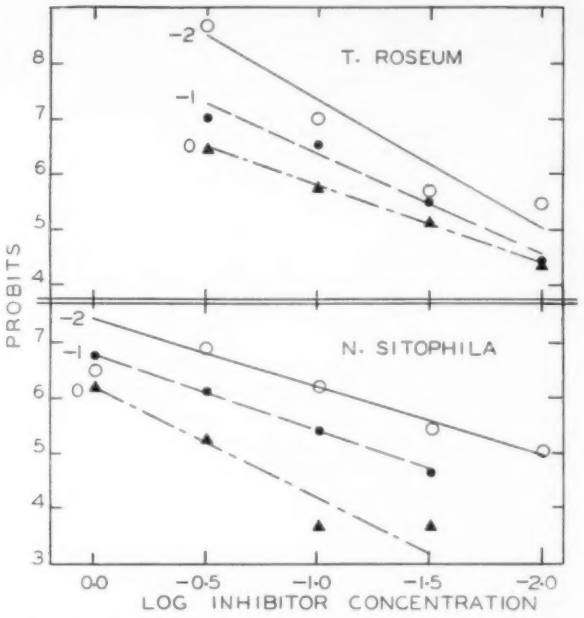

FIG. 2. Percentage reduction in infectivity, on 18 leaves of Kentucky Wonder bean plants, induced by adding varying dilutions of filtrate of *Trichothecium roseum* and *Neurospora sitophila* to constant amounts of SBM virus. Percentage reduction has been converted to probits and plotted as a function of the logarithm of inhibitor dilution.

dependent upon the concentration of inhibitor or on the assumption that the number of virus particles (n) or (t) is so dependent.

Experiments involving addition of a constant amount of inhibitor to various dilutions of virus.—A series of experiments was carried out, in each of which 18 leaves of Kentucky Wonder bean plants were inoculated with various dilutions of SBM virus containing a constant amount of inhibitor. Control virus solutions containing no inhibitor were inoculated onto the opposite leaves. Data from a typical experiment are shown graphically in figure 1, in which log (y/N) or log (Y/N′) is plotted as a function of log (vnx). N′ is the theoretical value of N when it is assumed that a constant amount of inhibitor depresses the value of N by a constant amount.

Although the data from the control virus solutions fit the curve of equation 1 rather poorly, those from the solutions containing a constant amount of inhibitor fit this equation almost as well if a lower value of N is taken. The latter do not fit the curve based on the assumption that a constant amount of inhibitor affects a constant amount of virus. Similar results were obtained by plotting the data according to equation 2. Thus, the data are consistent with the hypothesis that the inhibitor acts on the host.

Experiments involving addition of a constant amount of virus to various dilutions of inhibitor.—A series of experiments was carried out, in each of which 18 leaves of Kentucky Wonder bean plants were inoculated with solutions containing a constant amount of SBM virus and various dilutions of inhibitor. The control solutions, consisting of virus only, were inoculated onto the opposite leaves. From the numbers of lesions obtained, the percentage inhibition of virus infectivity was calculated. In figure 2 the data are plotted according to the equation $Y = mx + c$, where Y is the probit, or normal equivalent deviation, corresponding to the percentage inhibition and x is the log concentration of inhibitor (4). Since the data fit straight lines when plotted in this manner, they can be interpreted on either of two hypotheses: that the inhibitor affects the virus directly, or that it alters susceptibility of the host; provided the additional assumption is made that susceptibility of the virus, or susceptibility of the host cells, to the inhibitor is normally distributed.

Effect of diluting a mixture of virus and inhibitor.—An experiment was carried out wherein a $10^{-0.5}$ dilution of TM virus was mixed with a 10^{-1} dilution of inhibitor and twofold dilutions were made of the mixture. Each twofold dilution was then inoculated onto ten leaves of Golden Cluster bean plants. The opposite leaves were inoculated with corresponding dilutions of virus alone. The lesion counts and percentage inhibition are in table 1. The data show that, as a result of dilution of the mixture, infectivity is regained.

In attempting to interpret the data, the writers assumed that the inhibitor reacted with the virus in a manner to satisfy the mass law and applied the equilibrium equation of Gard (12). The plot thus obtained fit the theoretical curve very nicely, provided it was also assumed that the inhibitor was present in excess. However, identical results would be obtained if it is assumed that an equilibrium reaction takes place be-

TABLE 1. *Comparison of numbers of local lesions produced by serial dilutions of SBM virus with those produced by comparable dilutions of a mixture of virus and Trichothecium roseum filtrate*

Dilution	Number of lesions on 10 leaves of Kentucky Wonder bean plants		Percentage reduction
	Virus plus filtrate	Virus alone	
1	86	1307	93.42
1/2	245	1287	80.96
1/4	331	1437	76.96
1/8	711	1296	45.14
1/16	630	1116	43.55

tween inhibitor and host cells, or some constituent of host cells. Thus, the data do not provide for a choice between the hypothesis that the inhibitor acts on the virus and the hypothesis that it acts on the host.

CENTRIFUGATION STUDIES.—An experiment was carried out to determine whether or not virus present in a mixture with inhibitor could be separated by high-speed centrifugation. Two solutions, one containing virus and filtrate from *T. roseum* and the other containing virus alone, were adjusted to pH 7 by addition of 0.1 M phosphate buffer. The final dilution of virus and filtrate in the solutions was 10^{-1} and $10^{-1.5}$, respectively. These two solutions were then centrifuged at 11,000 r.p.m. for 90 min. in a Serval model SS-1a centrifuge. As will be seen later, the centrifugation was sufficient to sediment part, but not all, of the virus present in the solution. After centrifugation, six different fractions were prepared and tested for virus activity as follows:

A. The pellet obtained by centrifuging the virus alone was resuspended and restored to original volume by addition of buffer.
B. The pellet obtained by centrifuging the mixture of virus and inhibitor was resuspended and restored to original volume by addition of buffer.
C. The original virus preparation without the inhibitor.
D. The original virus and inhibitor mixture.
E. The supernatant remaining after the pellet of fraction B had been removed.
F. The supernatant remaining after the pellet of fraction A had been removed.

In carrying out activity tests a Latin square arrangement of test plant leaves was used. This arrangement involved three replications of six plants each of *Nicotiana glutinosa*. The plants were trimmed to exactly six leaves. The arrangement was such that each treatment appeared once on each plant and once in each leaf position in each replication.

Since analysis of variance was used in attempting to interpret the data, lesion counts on individual leaves were converted to logarithms. This was done because, as pointed out by Cochran (9), a logarithmic transformation of data offers an approximate correction where the error variance for treatment may be expected to depend upon the mean of the treatment. The logarithmically transformed data and the analysis of variance are in table 2.

TABLE 2. *Relative infectivities of fractions obtained by centrifuging TM virus and a mixture of TM virus and filtrate from Trichothecium roseum*

Sample	Sum of logarithms of lesion counts on six leaves of *N. glutinosa*			
	Replicate number			
	1	2	3	Total
A	11.82	11.84	11.53	35.19
B	9.80	11.15	10.53	31.48
C	13.05	12.66	13.64	39.35
D	6.07	5.23	6.91	18.21
E	4.74	5.63	4.76	15.13
F	12.10	13.09	12.87	38.06

Analysis of variance:

	Degrees of freedom	Sum of squares	Variance	Ratio
Replicates	2	0.1078	0.0539	2.07
Treatments	5	30.0458	6.0092	230.3 **
Treatments × replicates	10	0.6570	0.0657	2.52
Leaf positions	5	2.0695	0.4139	15.86**
Leaf positions × replicates	10	0.4991	0.0499	1.91
Plants	17	1.7428	0.1025	3.93**
Error	58	1.5132	0.0261	
	107	36.6352		

** Significant at the 0.01 level.

Variance between treatments is significant at the 0.01 level. However, it was desired to test the significance of the difference between specific pairs of treatments. The variance for the sum of 18 leaves is 0.02609×18. The standard error of the difference between pairs of treatments is therefore $\sqrt{0.02609} \times \sqrt{18} \times \sqrt{2}$ or 0.975. The ratio of the difference between treatments to the standard error of the difference, commonly referred to as student's "t," was calculated for various comparisons. The difference between B and D, A and B, A and F, F and E, and C and D all proved significant at the 0.01 level. The significant difference between lesion counts produced by the virus alone (C) and the virus-inhibitor (D) indicates that enough inhibitor was added to the mixture to obtain the desired inhibition. The difference between the pellet (A) and supernatant (F) from the virus solution shows that partial sedimentation of virus was accomplished by centrifugation at 11,000 r.p.m. for 90 min. However, the fact that the supernatant (F) was highly active shows that sedimentation of the virus was far from complete. The pellet (B) obtained by centrifugation of the virus-inhibitor mixture gave a significantly higher lesion count than that given by the original mixture (D), showing that partial separation of virus from the comparatively non-infectious mixture was accomplished. The difference in lesion counts between the pellet from the virus solution (A) and the pellet from the mixture (B) may be explained on the assumption that some of the inhibitor was sedimented along with the virus. The difference between the supernatant from the virus solu-

TABLE 3. *Relationship of time of contact between inhibitor and test plant leaves to reduction in SBM virus infectivity*

Test No.	Minutes of contact 0	5	10	20	40	80
1	7.8[a]	0.0	.....	38.2	51.1	.....
2	0.0	.....	18.7	10.2	57.6	76.2
3	55.3	65.2	.....	.....	.....	.....

[a] Percentage reduction in infectivity as determined by lesion counts on 12 leaves of Kentucky Wonder bean plants.

tion (F) and that from the mixture (E) is due to the presence of inhibitor in the supernatant from the mixture.

The results, which show that partial, if not complete, separation of virus from a comparatively noninfectious mixture of virus and inhibitor can be obtained by high-speed centrifugation, bring additional evidence for the view that the inhibitor acts upon the host plant to render it less susceptible to infection.

RELATIONSHIP OF TIME OF CONTACT BETWEEN INHIBITOR AND TEST-PLANT LEAVES TO REDUCTION IN SOUTHERN BEAN MOSAIC VIRUS INFECTIVITY.—Earlier experiments (14) showed that the time of contact between virus and inhibitor had no influence on percentage reduction of infectivity. It was therefore decided to see whether reduction of infectivity would be altered by allowing the inhibitor to remain in contact with the host for various lengths of time prior to inoculation with virus.

Leaves of Kentucky Wonder bean plants were washed 0, 5, 10, 20, 40, and 80 min. after spraying with a 10^{-1} dilution of inhibitor and were subsequently inoculated with a 10^{-1} dilution of SBM virus. Opposite leaves which served as controls were sprayed with pH 7 phosphate buffer and then washed and inoculated in a similar manner. The results are in table 3. They reveal an apparent correlation between the percentage inhibition of virus infectivity and the length of time the inhibitor was allowed to remain in contact with the leaf before being washed off. It is difficult to see how these results can be interpreted on the assumption that the inhibitor enters into a reaction with the virus. The results can, however, be interpreted on the hypothesis that the inhibitor renders the host cells insusceptible to the virus and that the reaction between host cells and inhibitor requires time for completion. Presumably the amount of inhibitor absorbed by the host cells determines the extent of inhibition.

EFFECT OF APPLICATION OF INHIBITOR TO LOWER SURFACE OF LEAVES.—Since results of previous lines of investigation suggested that the effect of the inhibitor was to render the host insusceptible, it seemed that it might be possible to render the host insusceptible in such a way that the virus and inhibitor do not come into actual contact. An attempt to do this was made by applying inhibitor to the lower surface of test plant leaves and then inoculating the virus onto the upper surface. Various dilutions of the inhibitor from *T. roseum* were sprayed uniformly onto the lower surface of 12 leaves of Golden Cluster and Kentucky Wonder bean plants. The spraying was carried out carefully so that none of the inhibitor got onto the upper surface. After a short interval, the upper surface of the same leaves was inoculated with a $10^{-0.5}$ or $10^{-1.5}$ dilution of TM virus or a 10^{-1} dilution of SBM virus, respectively. The number of lesions produced on these leaves was compared with the number produced on the opposite leaves whose lower surfaces had been sprayed with buffer solution and whose upper surfaces had been inoculated with equivalent concentrations of the same viruses. The results obtained are summarized in table 4.

TABLE 4. *Inhibition of TM virus and SBM virus infectivity obtained by application of filtrate to the undersurface of leaves*

Viruses	Percentage inhibition: Log dilution of filtrate −1.5	−1.0	−0.5
TM ($10^{-0.5}$)[a]	56	54	88
TM ($10^{-1.5}$)[a]	54	73	94
SBM (10^{-1})[b]	54	82	83

[a] Tested on Early Golden Cluster bean plants.
[b] Tested on Kentucky Wonder bean plants.

When the results are compared with those of previous experiments, it can be seen that the percentage inhibition was only slightly less when the inhibitor was applied to the lower surface and virus to the upper than when both inhibitor and virus were applied to the upper surface. The maximum inhibition obtained was 94 per cent for TM and 88 per cent for SBM virus. As in previous experiments, the percentage inhibition was dependent upon the inhibitor concentration and upon the virus concentration.

Seven layers of cells lie between the upper surface and the lower surface of a bean leaf. It would thus seem likely that inhibition of infection is not necessarily dependent upon direct contact between inhibitor and virus at or previous to the time of inoculation. However, it is not known whether or not the inhibitor penetrated from the lower cells to the upper ones without entering into a reaction with some cellular constituent. Nevertheless, the results bring additional evidence for the hypothesis that the inhibitory effect of *T. roseum* filtrate results from a change in host susceptibility.

INHIBITION WITH DIFFERENT HOST VIRUS COMBINATIONS.—To determine whether or not the host plant would influence the inhibitory effect of growth products of different fungi, filtrates of *Trichothecium roseum*, *Guignardia bidwellii*, and *Lambertella pruni* were mixed with tobacco mosaic, tobacco necrosis, and Southern bean mosaic viruses in the proportion of 8 parts of a 10^{-1} dilution of filtrate to one part of virus and one part of pH 7 molar phosphate buffer. In the control solution, sterile medium replaced the fungal filtrate. The inhibitory activity of each filtrate was determined by rubbing test and control solutions onto 12 opposite leaves of various host plants. The results are summarized in table 5. Although the mixture of tobac-

TABLE 5. *Comparative reaction of host species to viruses of tobacco necrosis, tobacco mosaic, and Southern bean mosaic treated with filtrates of (1) Trichothecium roseum, (2) Lambertella pruni, and (3) Guignardia bidwellii cultures*

Host	Percentage reduction in lesions								
	SBM virus filtrates			TM virus filtrates			TN virus filtrates		
	1	2	3	1	2	3	1	2	3
K. Wonder bean	99	96	100						
N. glutinosa				97	92	94	90	89	80
Necrotic-type tobacco				5	14	0	0	37	9
Golden Cluster bean				98	79	71	70	75	37
Turkish tobacco							0	0	36

co mosaic or tobacco necrosis virus and filtrate of *T. roseum* or *L. pruni* caused great reduction in the number of lesions on leaves of *N. glutinosa* and Golden Cluster bean, the same mixture caused little reduction on necrotic-type tobacco leaves. It can also be seen that the filtrate obtained from *G. bidwellii* did not inhibit infectivity for TN virus to the same extent it did TM virus. The same was also true for *T. roseum*. Furthermore, the filtrate of *G. bidwellii* caused 36 per cent inhibition of TN virus activity in Turkish tobacco, whereas that of *L. pruni* and *T. roseum* caused no inhibition at all.

If inhibition of virus activity were due to action of filtrate on the virus, one would not expect any large difference in percentage reduction in numbers of lesions from host to host. Therefore, the data suggest that action of the filtrates is primarily due to modification of host susceptibility, necrotic-type tobacco being less prone to such modification. The data further indicate that the inhibitory agent in the filtrate of *G. bidwellii* differs in some manner from that of *L. pruni* and *T. roseum*. There is at present no basis for judging whether this difference is quantitative or qualitative.

DISCUSSION.—Investigation of the mechanism of inhibition of virus activity by filtrates of *T. roseum* has provided five lines of evidence that it involves an effect on the host plant rather than a reaction between virus and inhibitory substance. The first of these concerns the quantitative change in virus activity of solutions in which the concentration of either the virus or inhibitor, or both, is varied. It has been shown that, if loss of activity is due to a reaction between inhibitor and virus, the assumption that a constant amount of inhibitor inactivates a constant amount of virus is untenable. On the other hand, the data do not permit a choice between the hypothesis that the inhibitor affects the virus and the hypothesis that it affects the host, if it is assumed either that the reaction between the host and the inhibitor, or between the virus and inhibitor, follows the mass law or that virus particles, or susceptible regions of the host, vary in sensitivity to the inhibitor.

The second line of evidence involves results which show that infectivity of virus in a mixture with inhibitor can be at least partially regained by high-speed centrifugation. The binding energy of a virus-inhibitor complex would need to be extremely weak to be broken by simple high-speed centrifugation.

The third line of evidence derives from the data which show that the longer the inhibitor is allowed to remain in contact with the host surface, up to 80 min., the greater is the inhibitory activity. It would appear that time is required for absorption of the inhibitor by the host cells or for a reaction between inhibitor and host cells.

The fourth line of evidence consists of the fact that the inhibitory substance is almost as effective in preventing infection on the upper leaf surface when applied to the lower surface as when applied to the upper. It is of course a remote possibility that the inhibitor is capable of traveling through seven layers of cells from the lower to the upper surface of the leaf and combining with virus without entering into a reaction with normal cellular constituents. If so, the reaction between inhibitor and virus must be assumed to be highly specific.

The fifth line of evidence derives from the fact that the inhibitory activity varies with the host plant. This was true for both tobacco mosaic virus and tobacco necrosis virus and for the inhibitory substances of *Guignardia bidwellii* and *Lambertella pruni*, as well as for that of *Trichothecium roseum*. Such results possibly can be interpreted on the assumption that the inhibitory substance differentially alters pathogenicity of a virus with which it reacts in a manner similar to that reported by Miller and Stanley (25) for tobacco mosaic virus treated with ketene, carbobenzoxy chloride, p-chlorobenzoyl chloride, or benzenesulfonyl chloride, and more recently by Chessin (7) for propagation of virus in plants deprived of nitrogen. They might also be interpreted by assuming, as Bawden (1) has, that some host plans are more capable than others of splitting inhibitor-virus complexes.

No one of the five lines of evidence is sufficient to prove that growth products of *T. roseum* inhibit virus infection by altering susceptibility of the host. When taken together, however, the evidence that such is the case becomes very strong. It is quite likely that other inhibitory materials act in a like manner although it is possible that still others may affect both the host and the virus.

SUMMARY

The inhibitory substance present in *Trichothecium roseum* was studied to determine the mechanism of its action in reducing infection of Southern bean mosaic, tobacco mosaic, and tobacco necrosis viruses. Five lines of evidence support the hypothesis that the inhibitory activity is due to alteration in host susceptibility. The first involved the nature of the quantitative change in activity of solutions in which the concentration of the inhibitor or the virus, or both, was varied. Second, the virus and inhibitor were partially sepa-

rated by high-speed centrifugation. Third, the longer the inhibitor was allowed to remain in contact with a leaf, up to 80 min., the greater was the inhibition. Fourth, the inhibitory substance was about as effective in preventing infection on the upper leaf surface when applied to the lower surface as when applied to the upper. Finally, the inhibitory activity was found to vary with the kind of host plant used.

DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PENNSYLVANIA

LITERATURE CITED

1. BAWDEN, F. C. Plant viruses and virus diseases. 3rd ed. 327 pp. Chronica Botanica Co. 1950.
2. ———, and N. W. PIRIE. Experiments on the chemical behaviour of potato virus X. Brit. Jour. Exp. Path. **17**: 64-74. 1936.
3. BLACK, L. M. Inhibition of virus activity by insect juices. Phytopath. **29**: 321-337. 1939.
4. BLISS, C. I. The calculation of the dosage-mortality curve. Ann. Appl. Biol. **22**: 134-167. 1935.
5. BRYAN, W. R., and J. W. BEARD. Host influence in the characterization of response to the papilloma protein and to vaccinia virus. Jour. Infec. Dis. **67**: 5-24. 1940.
6. CALDWELL, J. Factors affecting the formation of local lesions by tobacco mosaic virus. Proc. Roy. Soc. (London), B, **119**: 493-507. 1936.
7. CHESSIN, M. The effects of nitrogen deficiency on the properties of tobacco mosaic virus. Phytopath. **41**: 235-237. 1951.
8. CHESTER, KENNETH S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by immune sera. Phytopath. **24**: 1180-1202. 1934.
9. COCHRAN, W. G. Some difficulties in the statistical analysis of replicated experiments. Emp. Jour. Exp. Agr. **6**: 157-175. 1938.
10. DUGGAR, B. M., and J. K. ARMSTRONG. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Mo. Bot. Gard. **12**: 359-366. 1925.
11. FULTON, R. W. The sensitivity of plant viruses to certain inactivators. Phytopath. **33**: 674-682. 1943.
12. GARD, S. Purification of poliomyelitis virus. Acta Medica Scandinavica Supplement. 143. Uppsala. 1943.
13. GRANT, T. J. The host range and behavior of the ordinary tobacco mosaic virus. Phytopath. **24**: 311-336. 1934.
14. GUPTA, B. M., and W. C. PRICE. Production of plant virus inhibitors by fungi. Phytopath. **40**: 642-652. 1950.
15. HAMILTON, M. A. Further experiments on the artificial feeding of Myzus persicae Sulz. Ann. Appl. Biol. **22**: 243-258. 1935.
16. JOHNSON, J. Plant virus inhibitors produced by microorganisms. Science **88**: 552-553. 1938.
17. ———. Chemical inactivation and the reactivation of a plant virus. Phytopath. **31**: 679-701. 1941.
18. KLECZKOWSKI, A. Combination of potato virus X and tobacco mosaic virus with pepsin and trypsin. Biochem. Jour. **38**: 160-167. 1944.
19. ———. Combination between different proteins and between proteins and yeast nucleic acid. Biochem. Jour. **40**: 677-687. 1946.
20. ———. Interpreting relationships between the concentrations of plant viruses and numbers of local lesions. Jour. Gen. Microbiol. **4**: 53-69. 1950.
21. KUNTZ, J. E., and J. C. WALKER. Virus inhibition by extracts of spinach. Phytopath. **37**: 561-579. 1947.
22. LAUFFER, M. A., and W. C. PRICE. Infection by viruses. Arch. Biochem. **8**: 449-468. 1945.
23. LOJKIN, M., and C. G. VINSON. Effect of enzymes upon the infectivity of the virus of tobacco mosaic. Contrib. Boyce Thompson Inst. **3**: 147-162. 1931.
24. LORING, H. S. The reversible inactivation of tobacco mosaic virus by crystalline ribonuclease. Jour. Gen. Physiol. **25**: 497-505. 1942.
25. MILLER, G. L., and W. M. STANLEY. Derivatives of tobacco mosaic virus. II. Carbobenzoxy, p-chlorobenzoyl, and benzene-sulfonyl virus. Jour. Biol. Chem. **146**: 331-338. 1942.
26. MULVANIA, M. Studies on the nature of the virus of tobacco mosaic. Phytopath. **16**: 853-871. 1926.
27. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. 1. Some effects of trypsin. Phytopath. **24**: 1055-1085. 1934.
28. TAKAHASHI, W. N. A virus inactivator from yeast. Science **95**: 586-587. 1942.
29. ———. Properties of a virus inactivator from yeast. Science **104**: 377. 1946.
30. YOUDEN, W. J., H. P. BEALE, and J. D. GUTHRIE. Relationship of virus concentration to the numbers of lesions produced. Contrib. Boyce Thompson Inst. **7**: 37-53. 1935.

RESPONSE OF PEA VARIETIES TO DRY AND SLURRY METHODS OF SEED TREATMENT[1]

Donald J. deZeeuw and Axel L. Andersen[2]

Several investigators have shown that pea varieties, like varieties of other cultivated plants, differ in their susceptibility to plant diseases (14, 16, 17). Similarly, differential varietal responses to seed treatment have been found in peas (13), beans (2), flax (1), and sorghum (10). In addition to the above reactions, the authors observed another which was of interest. The investigations presented in this paper represent the results of experiments made in 1949 and 1950 on differences in varietal response of peas to seed treatment injury by Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide), and the conditions under which injury occurs. A preliminary report on these investigations has been made (4).

During the summer of 1949, it was noted that Alaska Wilt-Resistant peas were stunted as a result of the wet application of Ceresan M to the seed, whereas the variety Wisconsin Perfection was not injured. Examination of the roots of the affected plants disclosed a typical mercury-type injury very similar to that reported by Wilson (15) on peanuts and by Hull (6) on Alaska peas. Further studies were made in both greenhouse and field to obtain more information on this phenomenon.

MATERIALS AND METHODS.—For uniformity, all fungicide dosages used in the tests were calculated in ounces per 100 lb. of seed. For slurry treatments, either water or a 7 per cent solution of Methocel[3] was used, the latter product acting as both wetting agent and adhesive. The slurries were prepared by mixing 5 drops or less of the liquid with the required amount of fungicide. This was ample to coat completely 150 seeds. All slurry-treated seeds were dried thoroughly before storage prior to planting. The seed-treatment fungicides are in table 1.

In field experiments, the split plot design was used. There was some variation in field layout, but generally the first major split was for variety, the next for treatment chemical, and the last (single plot) for method of application. Each row (replicate) was 25 ft. long and rows were spaced 28 in. apart; 150 seeds were planted in each. All experiments were made with quadruplicate replication. Uniform depth of planting was achieved by using a Planet Jr. planter with an open hopper and gate, or a special space planter.

TABLE 1.—*Trade names and chemical composition of fungicides used in pea seed-treatment experiments*

Trade name	Manufacturer	Active ingredient
Arasan	DuPont	50 per cent tetramethylthiuram disulphide
Arasan SF	DuPont	75 per cent tetramethylthiuram disulphide
Ceresan M	DuPont	7.7 per cent ethyl mercury p-toluene sulfonanilide
Phygon XL	U.S. Rubber	50 per cent 2,3-dichloro-1,4-naphthoquinone
Spergon	U.S. Rubber	98 per cent tetrachloro-para-benzo-quinone

[3] Methocel, Dow Chemical Company brand of methyl cellulose.

The planting plan used in the greenhouse was similar to that used in the field. In this case 60 seeds (three 6-in. pots, each with 20 seeds) constituted a row. Varieties, fungicides, and methods of application were randomized to conform to the split-plot experimental design. The inoculum for damping-off consisted of naturally infested potting soil. After the first use, the infested soil was mixed with fresh soil for later tests according to the method of McCallan (11), with no noticeable impairment in damping-off potential.

Stand counts were used as a basis for treatment comparisons. In certain experiments the data included notes on length of vine and green weight as well as stand.

EXPERIMENTAL RESULTS.—Two varieties of peas, Alaska Wilt-Resistant and Wisconsin Perfection, were used in the first of the field experiments.[4] The treatments included Arasan at 2 oz./100 lb. or Arasan SF to give equivalent amounts of the active ingredient, Ceresan M at 1 2/3 oz./100 lb., Phygon XL at 1 2/3 oz./100 lb., and Spergon at 3½ oz./100 lb. The fungicides were applied dry or as water, Methocel, or "Z"[5] slurry. The seeds were planted approximately 3 weeks after treatment and the stand counts were made 25 days later (Table 2). In every row of Alaska treated with Ceresan M water slurry, the total emergence was low and the plants were stunted as compared to those from seeds receiving a corresponding dry treatment or left untreated. Furthermore, the variety Perfection did not have this particular injury.

An analysis of the data in table 2 indicated clearly that there was a differential response of the varieties to the various methods and kinds of seed treatment. Alaska, in general, showed only slight benefit from

[1] Accepted for publication September 4, 1951.
Contribution No. 52-1, Department of Botany and Plant Pathology, Michigan State College. Journal Article No. 1299 of the Michigan Agricultural Experiment Station.

[2] Respectively, Pathologist, Department of Botany and Plant Pathology, Michigan State College, and Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, and Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

[4] Seed used in the experimental work was furnished by the Ferry-Morse Seed Company.

[5] "Z" fungicide carrier. Product of Frank J. Zink Company. Used 1 part "Z" to 2 parts water.

TABLE 2.—*Average stands[a] of two varieties of peas treated with four seed-treatment fungicides by four methods of application*

Variety, and fungicide	Method of application			
	Dry	Water slurry	Methocel slurry	"Z" slurry
Alaska				
Arasan	143.5	142.2	143.0	143.0
Spergon	143.0	141.2	144.2	143.7
Phygon XL	141.5	142.7	141.2	141.7
Ceresan M	141.0	90.0	143.0	142.5
Check	136.2	132.7	136.5	130.2
Perfection				
Arasan	68.0	98.5	86.0	93.7
Spergon	66.2	76.2	79.7	65.2
Phygon XL	96.7	102.5	100.5	99.5
Ceresan M	86.0	111.0	104.2	74.7
Check	55.7	55.0	56.5	61.7

[a] 150 seeds per row.

L.S.D. at 5 per cent level within each horizontal row, 13.1.

L.S.D. at 1 per cent level within each horizontal row, 17.3.

fungicide treatments, whereas the stand of Perfection was increased. The results also show highly significant reductions in stand resulting from the treatment of Alaska seeds with Ceresan M water slurry, whereas the slurries of the other fungicides did not cause stand reductions. The variety Perfection, on the other hand, was benefited significantly by the seed treatments under nearly all conditions; in fact, the best treatment was the Ceresan M slurry which had injured the variety Alaska so severely. Apparently at this rate of application neither the Methocel nor "Z" slurries of Ceresan M caused significant reduction in stand of Alaska peas, even though the plants appeared somewhat less vigorous than those treated with dry Ceresan M.

Since Alaska pea was injured by the Ceresan M water slurry treatment, it was thought that other varieties might be injured in a similar manner and that susceptibility to injury could be correlated with some of the morphological characteristics of the seed. Two greenhouse tests were made to determine this during the ensuing winter months.

The first of the greenhouse tests included the varieties Dwarf Gray Sugar, a small, smooth-seeded pea with a mottled gray and brown seed coat; Large White Marrowfat, a large, smooth pea with a light-colored seed coat; Little Marvel, a wrinkled green pea; and Alaska Wilt-Resistant, a small, smooth, green pea. The above varieties were a fair representation of a large number of pea varieties as to size of seed and type of seed coat. All varieties were treated with Ceresan M, 2 oz./100 lb., applied as a dry powder, and in both water and Methocel slurries. Appropriate checks were included. The seed was treated, dried, and planted the following day. In this experiment, no injury occurred to any of the varieties from the treatments. On the contrary, Large White Marrowfat and Little Marvel were materially benefited by the seed treatment. Since the fungicide dosage was moderate and there was only a very short interval between the time of treating and planting, the experiment was repeated with a larger dosage and a longer interval between treating and planting.

TABLE 3.—*Average stand (60 seeds per row) of four pea varieties treated with Ceresan M seed protectant applied as a dust, water slurry, and Methocel slurry*

Variety	Method of application			
	Methocel slurry	Water slurry	Dry	Check (not treated)
Alaska	47.2[a]	5.2	57.0	39.0
Alderman	34.8	30.0	18.8	0.3
Dwarf Gray Sugar	42.2	45.8	43.0	42.8
Little Marvel	36.2	37.2	33.0	4.8
Average (all varieties)	40.1[b]	19.6	37.9	21.7

[a] L.S.D. (method × var.): 5 per cent level, 5.8.
1 per cent level, 7.8.

[b] L.S.D. (method): 5 per cent level, 3.5.
1 per cent level, 4.6.

Except for the variety Alderman, which was substituted for Large White Marrowfat, the same varieties were used in the second greenhouse experiment. Ceresan M, however, was applied at 4 instead of 2 oz./100 lb. seed. The same methods of application were used but the treated seed were stored 9 days before planting. Three weeks after planting, records were obtained on the stand of healthy plants, average height, and total green weight of the plants in each plot. The number of healthy plants did not include those plants which were badly lesioned or stunted. Since the data appeared to be closely correlated, only those pertaining to the average stand of healthy plants are presented (Table 3).

As far as the averages of all varieties for each method are concerned, the Methocel slurry was about equal to the dry method of application in the prevention of damping-off. Both the Methocel slurry and the dry treatments were significantly better than the nontreated control. The fact that the average stand for water slurry treatment was lower than the nontreated control was due almost entirely to the stand reduction in the Alaska variety. It is evident that Dwarf Gray Sugar is tolerant, Alaska intermediate, and Alderman and Little Marvel susceptible to damping-off. In the last three varieties there was a definite increase in stand as a result of the Ceresan M treatment, except for the water slurry treatment of the variety Alaska. The dry application was less beneficial than either slurry method on Alderman. In Alaska, both the dry and Methocel slurry applications gave stands superior to the control; and both methods, as well as the control, were significantly superior to the water slurry. In this experiment, the dry application was better than the Methocel slurry, indicating at least some injury by the Methocel method on Alaska pea.

Plants of the variety Alaska were more stunted by

the Methocel application than by the dry application of Ceresan M, even though the stand counts were only slightly lower (Fig. 1). The roots of these plants had evidence of mercury injury similar to that found on the plants which developed from seeds treated with water slurry, but in much less severe degree.

In Alaska pea seedlings from seeds treated with water-slurry Ceresan M and dry Ceresan M, the tap root was very much shortened and there was almost complete suppression of secondary roots on those plants which received the slurry seed treatment (Fig. 2). In general, the reduction in vine growth corresponds closely to reduction in root area. From the above results, there appeared to be no striking correlation between the morphological characteristics of the seeds and injury from the Ceresan M slurry treatment.

On the basis of the results obtained in the greenhouse and in the field the previous season, two field experiments were designed in 1950, to obtain further evidence as to the nature of the injury by Ceresan M and differences in varietal reaction. In the first of these, Alaska and Alderman were treated with Phygon XL (4 oz./100 lb.) and Ceresan M (4 oz./100 lb.) by the three methods used in the greenhouse. The seeds were treated 2 days before planting, and notes on stand were made at the end of 4 weeks. Before this time it was apparent that the Ceresan M water and Methocel treatments were injurious to Alaska and slightly injurious to Alderman (Table 4).

The variety Alderman responded to the seed treatments in the usual manner. Both fungicides, however, applied to the seed gave significantly better stands than no fungicide. Phygon XL would appear slightly better than Ceresan M in this case, probably because no injury occurred in the water and Methocel slurry applications. Alaska peas were injured by the Ceresan M water and Methocel slurry treatments, but more so by the water slurry. This confirms earlier observations in which Methocel appeared to protect the seed somewhat from mercury injury. Though the superiority of Phygon XL over the control on Alaska is not of the order of statistical significance, it is nevertheless believed that the increase was significant biologically, since nearly every row was uniformly better than its corresponding control row by the difference shown for the treatment average. Had the experiment consisted only of Phygon XL and check treatments, without the extremes brought in by Ceresan M, the difference would almost certainly have been statistically significant.

TABLE 4.—*Average stands (150 seeds per row) of two varieties of peas treated with two seed-treatment fungicides by three methods of application*

Variety	Method	Fungicide		
		Phygon XL	Ceresan M	Check
Alaska				
	Dry	134.8*	136.2	127.2
	Water slurry	140.0	26.2**	123.5
	Methocel slurry	138.0	51.8**	125.0
Alderman				
	Dry	115.0**	113.0**	47.8
	Water slurry	103.5**	97.2**	49.8
	Methocel slurry	116.2**	99.2**	38.5

* L.S.D. at 5 per cent level, 18.5; at 1 per cent level, 24.8.
** Difference between treatment and control significant at 1 per cent level.

The second field experiment was made to survey a number of pea varieties for other differences in reaction to Ceresan M. Twenty varieties were treated by the water slurry method at the rate of 4 oz./100 lb. of seed. In the field, each treated row was paired in random order with its untreated control. The seeds were planted 1 day after treating and notes on stand

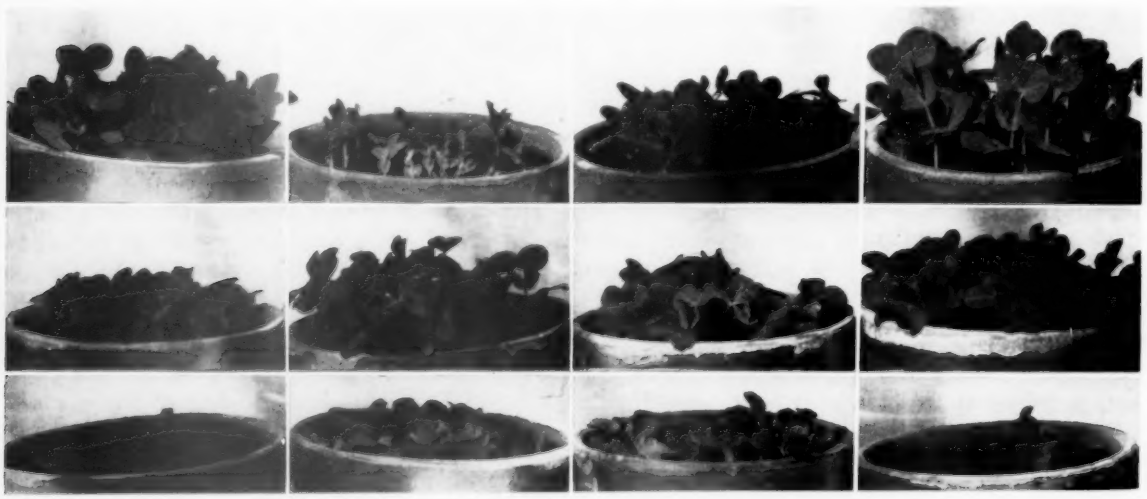

FIG. 1. Three varieties of peas treated with Ceresan M seed protectant by three methods of application. Rows: Top, Alaska Wilt-Resistant; middle, Dwarf Gray Sugar; bottom, Little Marvel. Columns: (Left to right) Ceresan M, dry; water slurry; Methocel slurry; and control, no treatment.

TABLE 5.—*Average stands (150 seeds per row) and stunting observed for 20 pea varieties treated with Ceresan M seed protectant applied as a water slurry*

Variety	Treated		Nontreated[a]
	Stand	Stunting	Stand
Large White Marrowfat	46.0**	Severe[b]	96.8
American Wonder	104.8	None	90.2
Bliss Everbearing	95.5	None	102.2
Dwarf Telephone	96.8	Slight	101.5
Giant Stride	88.0	Slight	78.5
Gradus	112.0*	None	92.2
Hundredfold	116.5	Slight	126.2
Improved Stratagem	94.5**	None	61.8
Laxtonian	105.2	None	108.0
Laxton's Progress	115.8**	None	90.5
Little Marvel	79.5**	Severe[b]	105.2
Miracle	98.8	Possible	109.5
Morse's Market	83.5	Severe	82.2
Oracle	123.8	Possible	114.2
Premium Gem	112.5*	Severe[b]	134.0
Thomas Laxton	104.8*	None	122.8
World's Record	85.2**	None	108.2
Wisconsin Perfection	104.2	Possible	115.5
Dwarf Gray Sugar	82.8**	Severe[b]	125.8
Melting Sugar	32.0**	Severe[b]	115.8

[a] No stunting.
[b] Significant reductions of stand were also caused by the treatment.
L.S.D., between treatments within varieties: *5 per cent level, 17.2. **1 per cent level, 23.0.

and varietal reaction were made approximately 4 weeks later (Table 5).

Of the 20 pea varieties tested, Gradus, Improved Stratagem, and Laxton's Progress appear to have benefited by seed treatment with Ceresan M water slurry. Five varieties, Large White Marrowfat, Little Marvel, Premium Gem, Dwarf Gray Sugar, and Melting Sugar were stunted severely and reduced in stand by the same treatment. The other varieties were intermediate in reaction; either the treatment made no difference in germination, or it did not produce significant stunting. There is a possibility that the treatment was injurious and still did not appear so in stand counts. This was evidenced by severe stunting in Morse's Market (Table 5), and yet the stand reduction, if any, caused by the treatment was no greater than that produced by damping-off in the nontreated rows. Other combinations of toxicity and damping-off in various degrees are also possible.

Because of space requirements and time limitations, it was not possible to include a nontoxic yet effective fungicide such as Phygon XL. If Phygon XL had been included it should have been possible to determine more easily whether toxicity was balanced by damping-off, or the Ceresan M was harmless. Possibly both explanations are correct in individual cases. The results did indicate, however, that there was a definite varietal selectivity as far as injury resulting from Ceresan M slurry treatment was concerned.

DISCUSSION.—The evidence presented emphasizes the necessity for caution in seed-treatment recommendations, particularly when potentially injurious materials such as the organic mercuries are to be used. Unfortunately, some of the best materials are just those with which one should be most careful. Even Arasan (12) may be injurious to seeds of several vegetables under some conditions.

The organic mercury seed fungicides are among the best available for many species, but at the same time they are the ones that need to be used most carefully. Leach (8) found that inclusion of organic mercury compounds in coated tomato seed resulted in reduced and retarded emergence. Colhoun (3) reported a decline in germination of wet-treated flax after storage of the treated seed. Several workers have reported evidence of mercury injury to peas under conditions similar to those reported here. For example, Haenseler (5) found that pea seeds treated with an or-

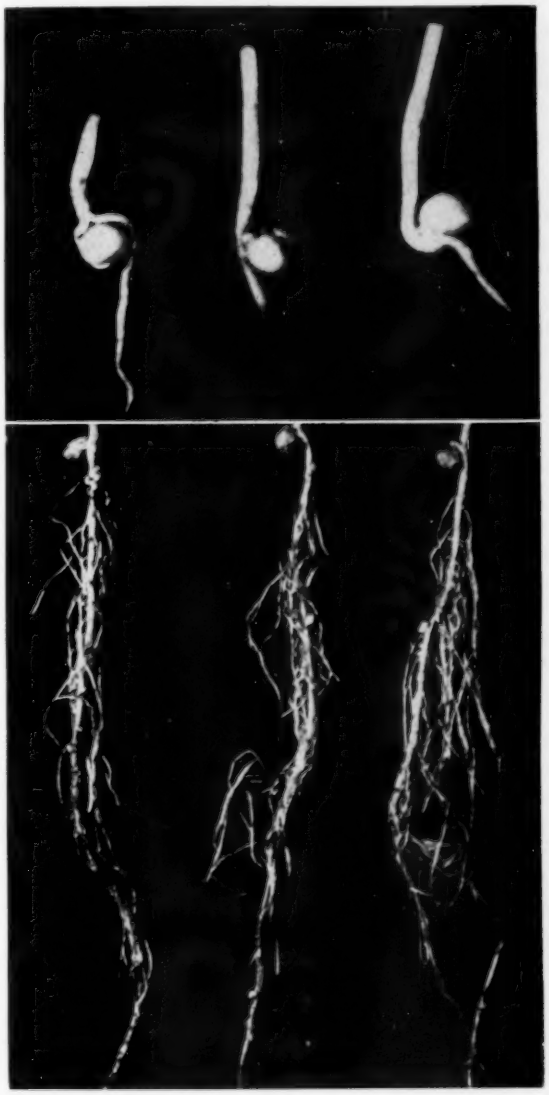

FIG. 2. Roots of Alaska Wilt-Resistant peas treated with Ceresan M seed protectant. Above (2/3 natural size): 4 oz./100 lb. seed in water slurry. Below (½ natural size): 4 oz./100 lb. seed used dry.

ganic mercury material in water gave stand reductions. He attributed the reduction to mechanical injury to the swollen seed. Unfortunately only dust treatments were tried in the part of his work in which he used Alaska peas. Jones (7) used, among other materials, Uspulun liquid and a 1:1000 $HgCl_2$ solution on three pea varieties including Alaska. He found no striking reduction of stand below the control. Leach and Smith (9) reported a mild cotyledon necrosis of peas as a result of dry Semesan treatment. The work most pertinent to results reported here was by Hull (6), who found that the tap roots of Alaska pea seedlings were noticeably more stunted by heavy dosages of a dry organic mercury seed dressing than were those of Gradus. No reductions of stand or of top growth and yield were observed, however.

The experimental evidence indicated that Ceresan M was one of the better seed treatments for peas when used as a dry dust at 2 oz./100 lb. of seed. Undoubtedly many of the pea varieties are injured by wet application of the material in excess, but in this work only one, Alaska, was found to be strikingly sensitive to moderate or light dosages.

The mechanism of injury is not known nor was any positive correlation between gross seed characteristics and mercury sensitivity found. It seems certain that most of the injury must occur at the time of treating, since dry-treated seed, even if planted in soil made wet to promote damping-off, did not show the same symptoms of toxicity. Further work to determine how one variety is made more sensitive than another has been begun.

SUMMARY

Pea varieties differ in resistance to soil-borne pathogens.

In general, the varieties most susceptible to damping-off responded best to seed treatment as evidenced by improved stand and vigor of plants.

All of the fungicides used, Arasan, Spergon, Phygon XL, and Ceresan M, were beneficial to susceptible peas under most conditions.

Pea varieties differed in their resistance to mercury injury caused by Ceresan M. Of all varieties tested, Alaska Wilt-Resistant was the most sensitive to injury when treated by moderate and light dosages of Ceresan M in water or Methocel slurry.

Three classes of peas were noted with respect to sensitivity to wet applications of Ceresan M: (a) varieties benefited by the fungicide; (b) varieties stunted and reduced in stand; and (c) varieties apparently not affected by the material, possibly because of lack of adequate control data.

The mechanism of mercury injury was not determined in the case of Alaska peas.

MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN

LITERATURE CITED

1. CHRISTENSEN, J. J., AND M. B. MOORE. Variability in response of flax to seed treatment. (Abstr.) Phytopath. **32**: 2. 1942.
2. COHN, ADAH E., AND DONALD J. DEZEEUW. Response of certain varieties of snap beans (Phaseolus vulgaris) to seed treatment. Mich. Agr. Exp. Sta. Quart. Bul. **32**: 386-401. 1950.
3. COLHOUN, J. The prevention of seed-borne diseases of flax. III. The dusting, short wet and fixation methods of seed disinfection in relation to storage of the seed. Ann. Appl. Biol. **32**: 34-37. 1945.
4. DEZEEUW, D. J., AND AXEL L. ANDERSEN. Response of pea varieties to dry and slurry methods of seed treatment. (Abstr.) Phytopath. **41**: 10. 1951.
5. HAENSELER, C. M. Effect of organic mercury seed treatments on germination and yield of peas. N.J. Agr. Exp. Sta. Ann. Rpt. **48**: 232-238. 1928.
6. HULL, R. Effect of environmental conditions, and more particularly of soil moisture upon the emergence of peas. Ann. Appl. Biol. **24**: 681-689. 1937.
7. JONES, L. K. Factors influencing the effectiveness of organic mercury dusts in pea seed treatment. Jour. Agr. Res. [U.S.] **42**: 25-33. 1931.
8. LEACH, L. D. Effects of combining fungicidal treatments and coating the seeds. (Abstr.) Phytopath. **38**: 916. 1948.
9. ———, AND PAUL G. SMITH. Effect of seed treatment on protection, rate of emergence, and growth of garden peas. Phytopath. **35**: 191-206. 1945.
10. LEUKEL, R. W., AND J. E. LIVINGSTON. Smut control in sorghum and effect of dust fungicides and storage on emergence. Phytopath. **35**: 645-653. 1945.
11. MCCALLAN, S. E. A. Evaluation of chemicals as seed protectants by greenhouse tests with peas and other seeds. Contrib. Boyce Thompson Inst. **15**: 91-117. 1948.
12. MCKEEN, C. D. Arasan as a seed and soil treatment for the control of damping-off in certain vegetables. Sci. Agr. **30**: 261-270. 1950.
13. MCNEW, G. L. Varietal reaction of peas to seed treatment. N. Y. (Cornell) Agr. Exp. Sta., 63rd Ann. Rpt. 1944. p. 33.
14. WADE, B. L., AND W. J. ZAUMEYER. Varietal reaction of pea to a virus from alsike clover. Phytopath. **28**: 505-511. 1938.
15. WILSON, COYT. Peanut seed treatments. U.S. Dept. Agr., Pl. Dis. Rptr. **34**: 87-95. 1950.
16. ZAUMEYER, W. J. Reaction of pea varieties to Septoria pisi. Phytopath. **32**: 64-70. 1942.
17. ———, AND B. L. WADE. Varietal reaction of pea to pea-streak virus 1. Phytopath. **27**: 1009-1013. 1937.

Thiolutin as a Possible Inhibitor of Fire Blight.[1] A. E. MURNEEK. During the past few years fire blight has become a widespread and extremely serious disease of the apple and related species in Missouri and adjoining territory. After attempts to curtail the ravages of the causal organism, *Erwinia amylovora*, with Bordeaux, Dithane Z-78, Kolofog, and Bioquin sprays without much success, two antibiotics, Streptomycin and Thiolutin, were tested in the spring of 1951.[2]

Apple trees of the susceptible Jonathan variety, on which fire blight had been endemic for the past few years, were used for the test. The treated and control trees were in adjoining rows. Both of the materials were applied in aqueous solution by means of a power sprayer. At the time of spraying (May 5) approximately 90 per cent of the flowers were open. A "late bloom" spray was not applied because of lack of material.

Results were determined on July 6, about 2 months after spraying. With Thiolutin, 19 gm. per 100 gal. water, the average number of infections per tree was 48; with Streptomycin, 10 gm., 94 infections. In the untreated controls, infections averaged 224 per tree.

Streptomycin is a familiar antibiotic. Thiolutin,[3] isolated from certain strains of *Streptomyces albus*, and distinct from the related Actinomycetin, was so named because of a characteristic yellow color. It seems to be active against many organisms, including Gram positive and Gram negative bacteria and numerous fungi, hence its possible usefulness in phytopathology.

Whether or not Thiolutin may be of real value as a protection against fire blight manifestly cannot be definitely ascertained by a small-scale preliminary test, involving four trees in each group. Apparently it is more potent for this purpose than Streptomycin.—University of Missouri, Columbia, Missouri.

A Rapid Method for Determining Oak Wilt.[1] H. L. BARNETT. The usual technique for the determination of oak wilt involves planting chips from a suspected tree on malt extract or potato glucose agar. Under these conditions positive identification of *Chalara quercina*, a slow-growing fungus, may not be possible for 2–3 weeks, when the typical conidiophores and endoconidia are produced.

During a preliminary study of the factors influencing sporulation of this fungus, methods were discovered which markedly reduce the time required for identification. The methods are presented at this time in order to aid those who are now carrying on routine culture and identification work on oak wilt. They are based, in part, on the unusual and characteristic ability of *C. quercina* to sporulate readily in submerged liquid culture.

When fresh chips (bark removed) from oak-wilt trees are placed directly into tubes of a liquid medium, the characteristic conidiophores and conidia are formed within 5 days at 25° C. Apparently the medium need not be of any specific composition, for rapid sporulation has occurred in dilute malt extract, potato extract, sucrose-asparagine, glucose-casein hydrolysate, and even in distilled water. Sporulation was least in distilled water and much greater in glucose-casein hydrolysate than in any other medium. The optimum amount of sugar for quick sporulation has not been determined, but 5 gm. per liter has been satisfactory. Since *C. quercina* is deficient for biotin, this vitamin must be added to synthetic media.

It is not known at present what complicating effects may arise from the presence of rapidly growing contaminants, but since few fungi are able to sporulate in submerged liquid media their presence may not be serious. However, an alternate procedure may be used which would eliminate most of the other fungi. Oak chips may be cultured on agar for 4 or 5 days until some mycelium is formed. Those having the appearance of *C. quercina* may then be transferred to tubes of a dilute liquid medium and incubated for 2 days at or slightly below 25° C. At this time microscopic examination should reveal numerous conidiophores and conidia. The conidiophores formed in submerged culture are typical in shape but are hyaline and shorter than those formed aerially, being 20–40 μ long.—Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.

Influence of Littleleaf on Quality of Shortleaf Pine Seed. L. W. R. JACKSON.[1] Quality of seed crop is important in the establishment of an adequate supply of seedlings by the process of natural reproduction. Seed quality is affected by such factors as site conditions, age of the tree, insects, and diseases. In the Piedmont region, the prompt establishment of an adequate crop of seedlings is of major importance in the management of pine forests to avoid conversion of the area to low-quality hardwoods. Prompt reseeding is of particular importance in stands of shortleaf pine (*Pinus echinata* Mill.) in which a number of trees are killed annually by littleleaf disease. This report presents

[1]Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station, Columbia, Missouri. Journal Series No. 1265.

[2]The writer wishes to express his gratitude to Merck & Co. for supplying Streptomycin and to Charles Pfizer & Co., for supplying Thiolutin.

[3]Tanner, Fred W., John A. Means, J. W. Davisson, and Arthur R. English. Thiolutin, an antibiotic produced by certain strains of Streptomyces albus. Charles Pfizer and Company, New York (in press).

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 444.

[1]Professor of Silviculture, Forestry School, University of Georgia.

TABLE 1.—*Summary of data on quality of cones and seed from healthy and diseased shortleaf pines grown on comparable sites*[a]

Lot No.	Number of seeds per pound	Percentage of seeds filled	Average cone wt. (in gm.)	Percentage of total germination
1	66,240	84	6.6	74.0
2	67,260	72	3.3	62.0
3	91,717	57	2.1	24.5
4	108,095	24	2.5	4.0

[a]Tree condition: Lot 1, healthy; lot 2, early stages of littleleaf; lot 3, moderate, and lot 4, severe littleleaf.

the results obtained from a study of the effect of littleleaf on the quality of shortleaf pine seed.

The cones from littleleaf and healthy trees were air dried and the seeds were extracted by hand. Twenty-five cones of each lot were dried to a constant weight at 110° C. Total germination was based on 400 seeds from each lot. A cutting test was made of 200 seeds of each lot. The seeds were germinated in flats containing a mixture of peat moss and sand, in a room where the daily temperature varied from 65° to 80° F. The data in table 1 were obtained from the study.

These data indicate that an increase in the severity of littleleaf caused a marked decrease in the size of cones (Fig. 1) as well as in viability of the seed. The dry weight of cones from trees with moderate or severe littleleaf was only 39 per cent of that of healthy trees. Seed size was greatly reduced, as shown by the increase in the number of seeds per pound. Viability, as shown by the percentage of filled seed, dropped consistently from 84 per cent for healthy seed to 24 per cent for seed from trees with severe littleleaf.

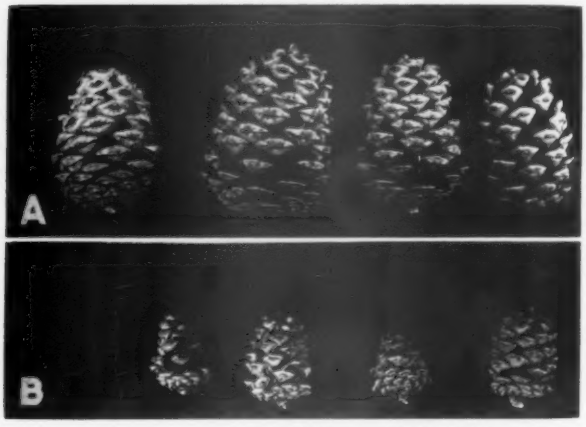

FIG. 1. Cones from (A) healthy and (B) littleleaf trees of shortleaf pine grown on comparable sites.

Of particular importance, however, was the drastic reduction in germinative capacity, as shown by the percentage of total germination, which dropped from 74 per cent for seed from healthy trees to only 4 per cent for seed from trees with severe littleleaf. Further evidence of low viability was shown by the minimum number of days from planting to start of emergence. Seedlings from seed of healthy trees usually start emerging about 12 days after planting, reaching a peak in about 21 days. By comparison, seedlings from seed of trees with moderate and severe littleleaf began to emerge after 60 and 125 days, respectively. Germination of seed from trees with severe littleleaf was not completed until 190 days after planting.

Similar results were obtained by Buchanan[2] in a study of the symptoms of the littleleaf disease. According to his data, the average fresh weight of cones from littleleaf trees was 3.96 as compared with 8.39 gm. for healthy trees. Seed size was reduced from 129 per gm. for healthy trees to 296 per gm. for littleleaf trees. The amount of filling was reduced from 58.4 per cent for healthy trees to 43.2 per cent for littleleaf trees. At the end of 90 days, the germination was only 23.2 per cent for littleleaf seed as compared with 48.4 per cent for healthy seed. Buchanan reported, however, that the filled seed from both the diseased and healthy trees showed similar germination trends during the first 40 to 45 days of the test.

According to the results of this study, the littleleaf disease causes marked deterioration of quality of shortleaf pine seed. It is known that the quality of tree seed is closely related to the accumulation of reserve foods produced by the process of photosynthesis. Therefore, it appears that the poor quality of seed produced by littleleaf trees is related to factors that affect the synthesis of carbohydrates. Shortleaf pines with moderate or severe littleleaf must be regarded as low-quality seed trees in the stand.—School of Forestry, University of Georgia, Athens, Georgia.

[2]Buchanan, T. S. Characteristics of the little-leaf disease of Southern pines. Thesis. Yale University. 1943.